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Feature Article

Cerebral Hypoxia: Some New Approaches and Unanswered Questions

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The apoplexy of Hippocrates' time remains with us today, unchanged and untreated. We call this syndrome of acute brain damage "stroke"; we know that it most commonly reflects localized tissue hypoxia attributable to reduced blood flow (ischemia). Focal hypoxia-ischemia also occurs in such contexts as traumatic insults, or cerebral hemorrhages, while global hypoxia-ischemia occurs in cardiac arrest, near-drowning, and carbon monoxide poisoning. The centuries since Thomas Willis, Johann Wepfer, and Giovanni Morgagni have brought precise definition of cerebral vascular anatomy and the neurological consequences of focal brain lesions, permitting full comprehension of functional deficits; we can prognosticate with sad accuracy. But the medical management of stroke patients in 1990 is still the management of symptoms and associated conditions. Despite its status as a major worldwide cause of death and disability, we are no more able than Hippocrates to treat cerebral hypoxia itself.

Nevertheless, hope for the development of effective therapy has endured, and in the last few years has been encouraged by the emergence of some promising strategies for reducing the brain's intrinsic susceptibility to hypoxic insults. These tissue-level approaches, sometimes referred to as "parenchymal" approaches to distinguish them from other strategies aimed at influencing blood flow, are based on recent information suggesting that central neurotransmitter mechanisms, especially those related to the excitatory neurotransmitter glutamate, may play an important role in the pathogenesis of hypoxic neuronal death (Meldrum, 1985; Rothman and Olney, 1986; Choi, 1988b). In this essay I will comment on the possibility of new therapies for cerebral hypoxia directed at glutamate-mediated injury mechanisms, and will briefly mention some other potential approaches.

Glutamate and hypoxic neuronal injury

The brain is critically dependent on its blood flow for a continuous supply of oxygen and glucose. The oscillations of the electroencephalogram cease within seconds of cardiac arrest, and only a few minutes of severe ischemia can induce the selective degeneration of certain neuronal populations, including pyramidal neurons in the CA1 region of the hippocampal formation, striatal medium-sized neurons, neocortical neurons in layers 3, 5, and 6, and cerebellar Purkinje neurons (Brierley, 1976). More sustained ischemia, compatible with the patient's survival only if localized, can produce infarction: a region of pannecrosis involving neurons, glia, and endothelial cells.

The special vulnerability of certain brain neurons to hypoxic-ischemic injury was recognized by Vogt and Vogt (1937), who hypothesized that it was explained by intrinsic neuronal properties. Subsequent efforts to identify the parenchymal determinants of hypoxic neuronal injury focused on general metabolic derangements: in particular, the gap between oxygen demand and supply, and the resulting energy deficit. However, the simple idea that an energy deficit directly causes neuronal death conflicts with available data. Most neurons can survive periods of complete ischemia sufficient to reduce phosphocreatine and ATP to negligible levels; and, paradoxically, incomplete ischemia causes more neuronal death than complete ischemia (Siesjö, 1981). Furthermore, pharmacological reduction of cerebral metabolism by means of barbiturates does not consistently protect against neuronal death in experimental or clinical brain ischemia (Safar, 1980; Nussmeier et al., 1986; but see Grotta, 1987). More recent evidence has suggested that the parenchymal approach to the therapy of cerebral hypoxia can be improved by attending specifically to brain excitatory synaptic mechanisms.

The pathogenesis of hypoxic neuronal injury was first linked to synaptic transmission by Kass and Lipton (1982) and Rothman (1983), who found that elevating extracellular magnesium reduced the vulnerability of hippocampal neurons *in vitro* to anoxic insult. Attention was focused on the role of glutamate-mediated synaptic transmission by reports that glutamate antagonists reduced injury both *in vitro* (Rothman, 1984) and *in vivo* (Simon et al., 1984). Injury reduction has now been reported with glutamate antagonists [especially those effective against *N*-methyl-D-aspartate (NMDA) receptors (see below)] in several models of focal brain ischemia, as well as in models of hypoglycemia, prolonged seizures, and mechanical trauma (Choi, 1988b, Albers et al., 1989).

Most likely, these protective effects of glutamate antagonists reflect the blockade of neuronal death caused directly by glutamate overexposure. As discovered by Lucas and Newhouse (1957), and Olney (1969), excessive exposure to glutamate or related excitatory amino acids can kill central neurons, a process Olney labeled "excitotoxicity." Microdialysis measurements have indicated that extracellular levels of glutamate are increased during hypoxia (Benveniste et al., 1984; Globus et al., 1988), likely reaching levels sufficient to kill briefly exposed cultured neurons (Choi, 1988b). In addition, lesioning the glu-

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tamate-mediated excitatory inputs to hippocampus reduces hypoxia-induced selective neuronal loss (Johansen et al., 1986; Onodera et al., 1986).

The hypothesis that glutamate neurotoxicity might contribute to the pathogenesis of hypoxic-ischemic neuronal damage tied together 2 previous ideas: (1) the idea of Van Harreveld (1959), Hansen (1985), and others that the electrophysiological changes accompanying hypoxia are similar to those accompanying spreading depression, another phenomenon probably mediated by NMDA receptors (Mody et al., 1987); and (2) the idea that selective neuronal injury after hypoxic insult is an active process, becoming apparent only after a delay of 48–72 hr (Kirino, 1982; Pulsinelli et al., 1982). Furthermore, the possibility that glutamate neurotoxicity might itself be mediated by an influx of extracellular Ca^{2+} and the formation of free radicals (Choi, 1988b; also see below) may help account for the previously postulated participation of Ca^{2+} and free radicals in the pathogenesis of hypoxic neuronal injury (Meldrum et al., 1985; Siesjö, 1989; Siesjö and Bengtsson, 1989).

Several signaling systems other than those mediated by glutamate or related compounds may additionally influence hypoxic neuronal injury. Stimulation of adenosine A_1 receptors reduces hypoxic neuronal injury both *in vivo* (Evans et al., 1988; von Lubitz et al., 1988) and *in vitro* (Goldberg et al., 1988), whereas adenosine antagonists increase injury (Wieloch et al., 1985; Rudolphi et al., 1987). In forebrain ischemia, toxic lesions of the locus coeruleus aggravate hippocampal and cortical brain damage (Blomqvist et al., 1985; Davis et al., 1987), while administration of a mixture of adrenaline and noradrenaline ameliorate it (Koide et al., 1986). Finally, ablation of the substantia nigra attenuates striatal ischemic injury (Globus et al., 1987).

These effects are likely to be mediated in part by alterations in the glutamate transmitter system, although other alterations, for example in blood flow or postsynaptic neuronal membrane properties, may also participate. Adenosine in particular can inhibit glutamate release (Dolphin and Archer, 1983); norepinephrine may do the same (Dunlap and Fischbach, 1981; Crowder and Bradford, 1987); and dopamine may inhibit electrical stimulation-evoked glutamate uptake (Kerkerian et al., 1987).

Blockade of glutamate toxicity

The hypothesis that excitotoxicity is an important cause of neuronal death in cerebral hypoxia-ischemia raises several possibilities for therapeutic intervention. Observations on cultured neurons suggest that intense exposure to glutamate induces 2 events: acute neuronal swelling dependent on extracellular Na^+ and Cl^-, and delayed neuronal disintegration dependent on extracellular Ca^{2+} (Choi, 1988a). By analogy to long-term potentiation, these events may reflect a sequence of 3 stages, each perhaps amenable to specific therapeutic interference (Choi, 1990).

Stage 1. Induction

This stage consists of the initial events leading to overstimulation of glutamate receptors, and consequent immediate intracellular derangements. Glutamate activates both NMDA and non-NMDA (kainate and AMPA/quisqualate) type receptors, which are linked to Na^+ and K^+ channels (Watkins and Olverman, 1987; Collingridge and Lester, 1989) and mediate acute neuronal swelling. NMDA receptors are particularly important in mediating subsequent delayed neuronal disintegration (Choi, 1988a; Michaels and Rothman, 1990), perhaps because their channels carry Ca^{2+} as well (MacDermott et al., 1986). Glutamate also activates a quisqualate-preferring metabotropic receptor (Eccles and McGeer, 1979), which induces the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) to generate the second messenger's inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (Collingridge and Lester, 1989).

Glutamate receptor overstimulation of neurons thus induces a solute derangement consisting of an accumulation of intracellular Ca^{2+}, Na^+, Cl^-, water, IP_3, and diacylglycerol, as well as of a depletion of intracellular K^+. Although this intracellular image of glutamate receptor overstimulation is potentially lethal, it precedes the occurrence of irreversibly lethal events. Evidence that induction of the derangement can be uncoupled from cell death is provided by the observation that virtually all cortical neurons destined to die after overexposure to glutamate can be rescued by a 20 min incubation in Na^+- and Ca^{2+}-free solution (Hartley and Choi, 1989). Possibly, this treatment draws Na^+ and Ca^{2+} back out of neurons before irreversible harm has occurred.

Reducing hypoxic excitotoxic induction might be accomplished most easily postsynaptically by antagonizing NMDA receptors. Fortunately, NMDA receptors have many potential antagonist target sites, including: (1) the agonist-binding site itself; (2) the glycine-binding site; (3) a zinc-binding site; (4) channel-blocking sites defined by phencyclidine, magnesium, or zinc; (5) a polyamine-binding site; and (6) regulatory sites sensitive to changes in pH, phosphorylation, or oxidation (Collingridge and Lester, 1989; Choi, 1990). Antagonist compounds are available, most of them interacting at the agonist-binding site (Watkins and Olverman, 1987; Lehmann et al., 1988), the phencyclidine-binding site (Kemp et al., 1987), or the glycine-binding site (Johnson and Ascher, 1987; Kemp et al., 1988).

Although NMDA receptors may play a key role in the mediation of hypoxia-induced excitotoxicity, concurrent activation of non-NMDA receptors or the metabotropic glutamate receptor may augment injury (Frandsen et al., 1989; also see below). Furthermore, certain neuronal subpopulations, such as cortical neurons containing the enzyme NADPH-diaphorase (Koh and Choi, 1988) or parvalbumin-like immunoreactivity (Weiss et al., 1990), may be unusually vulnerable to non-NMDA receptor-mediated injury. Antagonists for non-NMDA receptors have been described (Sheardown et al., 1990). The pharmacology of the metabotropic receptor is not well defined, but 2-amino-3-phosphonopropionate is a possible antagonist (Schoepp and Johnson, 1989).

Reduction of excitotoxic induction might also be accomplished presynaptically, by reducing glutamate release from axon terminals, or by facilitating glutamate clearance from synaptic clefts. Attainment of the former goal of reducing glutamate release will be aided by ascertaining the extent to which glutamate release in hypoxia-ischemia depends on glutamate synthesis, neuronal activity, Ca^{2+}-dependent vesicular release, or reversal of carrier-mediated uptake. Currently promising approaches to the reduction of glutamate release stratagem include stimulation of adenosine A_1 receptors (see above), indirect inhibition of glutamate synthesis with methionine sulfoximine (Swanson et al., 1990), and hypothermia (Busto et al., 1989).

Stage 2. Amplification

This stage consists of the postsynaptic cascades that augment the intensity of initial derangements, and promote the spread

Table 1. Some enzymes possibly linking glutamate receptor activation to the lasting enhancement of excitatory synaptic efficacy and excitotoxicity

Enzyme	Activators	Possible result
Protein kinase C	Ca^{2+}, DAG	increased glutamate release
		increased glutamate response
		increased VGCC conductance
		decreased afterhyperpolarization
		decreased Cl^- conductance
CaM kinase II	Ca^{2+}, CaM	increased glutamate response
		increased glutamate release
Calcineurin	Ca^{2+}, CaM	decreased $GABA_A$ receptor-mediated response
Calpain I	Ca^{2+}	breakdown of cytoskeleton →
		remodeling of postsynaptic spines
		increased excitatory synaptic efficacy
Phospholipase A_2	Ca^{2+}	formation of arachidonic acid and metabolites →
		increased glutamate release
		decreased glutamate uptake
		increased glutamate response

Abbreviations: DAG, diacylglycerol; VGCC, voltage-gated Ca^{2+} channel; CaM, calmodulin; CaM kinase II, Ca^{2+}/calmodulin-dependent protein kinase II.

References: Chin et al., 1985; Kaczmarek, 1987; Piomelli et al., 1987; Lynch et al., 1988; Bazan, 1989; Kennedy, 1989; Malenka et al., 1989; Melloni and Pontremoli, 1989; also see references listed in the text.

of excitotoxicity to other neurons. The key derangement may be a buildup of intracellular Ca^{2+}; the role of elevations in Na^+, IP_3, and diacyglycerol may derive largely from their enhancement of this accumulation. Three main events may occur.

First, the Ca^{2+} initially entering through the NMDA receptor-gated channel may be augmented by other sources of Ca^{2+} influx (Choi, 1988a), including voltage-gated Ca^{2+} channels, reverse operation of the Na^+-Ca^{2+} exchanger (Nachsen et al., 1986), some cation channels activated by Ca^{2+} (Partridge and Swandulla, 1988), channels activated by membrane stretch (Yang and Sachs, 1989), or membrane leak conductances. Furthermore, IP_3 will induce the release of Ca^{2+} from intracellular stores (Berridge and Irvine, 1989).

Second, stimulation of glutamate receptors and elevation of intracellular free Ca^{2+} activates several enzyme families—including C kinases, calmodulin-regulated enzymes, calpains, and phospholipases—which may orchestrate the long-term enhancement of excitatory synaptic efficacy and circuit excitability (Table 1). Specific alterations may include increased glutamate release (Diaz-Guerra et al., 1988; Malinow et al., 1989; Williams et al., 1989), decreased glutamate uptake (Yu et al., 1987; Barbour et al., 1989), potentiation of postsynaptic glutamate receptor-mediated responses (Kimura et al., 1985, Malenka et al., 1989), increased Ca^{2+} current through voltage-gated channels (Strong et al., 1987; Connor et al., 1988), and reduction of $GABA_A$ receptor function (Stelzer et al., 1988). The long-term enhancement of excitatory synaptic efficacy, and hence possibly also of excitotoxicity, might also be promoted by early-immediate gene expression (Cole et al., 1989; Morgan and Curran, 1989; Szekely et al., 1989).

Third, some combination of increased neuronal activity, tonic depolarization, Ca^{2+} accumulation, and cell membrane damage may lead to a secondary efflux of endogenous glutamate stores and continued stimulation of glutamate receptors—a positive-feedback loop that likely accounts for observations that NMDA antagonists applied after the end of exposure to toxic levels of glutamate or NMDA can still reduce resultant neuronal death (Rothman et al., 1987; Foster et al., 1988; Hartley and Choi, 1989).

Protective strategies operative at the level of amplification might include blockade of additional Ca^{2+} influx, blockade of Ca^{2+} release from intracellular stores, or interference with the specific mechanisms coupling glutamate receptor stimulation to lasting enhancements of excitatory synaptic efficacy. A particularly attractive target for such blockage may be L-type voltage-gated Ca^{2+} channels, as clinical tests of dihydropyridine antagonists have shown evidence of protective efficacy in ischemia (Grotta, 1987; Uematsu et al., 1989). Another promising target may be protein kinase C, which catalyzes phosphorylation of many proteins and which has been implicated in both presynaptic and postsynaptic changes associated with long-term potentiation (see above). Protection against both glutamate toxicity and ischemic injury has been reported for gangliosides, which can inhibit the membrane translocation of protein kinase C (Favaron et al., 1988; Komatsumoto et al., 1988). Reduction of ischemic brain injury has also been reported for other protein kinase C inhibitors (Kogure, 1990).

The benefits of methods for reducing excitotoxic amplification may depend on the magnitude of the initial NMDA receptor-mediated Ca^{2+} influx. If the initial Ca^{2+} influx is large, reflecting intense activation of large numbers of NMDA receptors, then there may be little need for further amplification to reach toxic levels of intracellular Ca^{2+} accumulation. A workable strategy in such cases might be direct reduction of effective Ca^{2+} accumulation: increasing the extrusion, sequestration, or binding of intracellular free Ca^{2+}. For instance, injury of dentate hilar cells induced by excessive perforant pathway stimulation can be reduced by the intracellular administration of a calcium chelator (Scharfman and Schwartzkroin, 1989).

Stage 3. Expression

This stage consists of the final events responsible for cell disintegration, possibly triggered by high levels of intracellular Ca^{2+} (Cheung et al., 1986; Choi, 1988a; Siesjö and Bengtsson, 1989).

The activation of degradative enzymes and the generation of free radicals may be particularly important factors in disintegration.

Neurons contain high levels of the protease calpain I, which may undergo autoproteolytic activation in the presence of high concentrations of free Ca^{2+}, and catalyze excessive proteolysis (Melloni and Pontremoli, 1989). Hippocampal neurons exposed to kainate or NMDA exhibit breakdown of spectrin and the microtubule-associated protein, MAP2, which correlates well with subsequent neuronal degeneration (Siman et al., 1989). Intracellular free Ca^{2+} may also activate phospholipase A_2, which can degrade membrane phospholipids, likely contributing to catastrophic cell or organelle membrane failure, and generating arachidonic acid. Arachidonic acid may have potentiating effects on excitatory synaptic transmission (Table 1); its further metabolism may produce oxygen-free radicals (see below) and delay deleterious effects on tissue blood flow (see next section). Finally, Ca^{2+}-activated endonucleases can induce DNA fragmentation, a process that occurs in programmed cell death (Nicotera et al., 1989), and which may be the ultimate limit for subsequent recovery.

Ca^{2+}-induced formation of arachidonic acid can be expected to enhance catabolic steps leading to the formation of substantial amounts of oxygen free radicals (Chan et al., 1985; Siesjö, 1989). Glutamate may also inhibit neuronal cystine uptake, leading to reduced glutathione synthesis and diminished capacity to scavenge free radicals (Murphy et al., 1989). An indication that free radical-induced damage, including lipid peroxidation, may be a key mediator of glutamate neurotoxicity, is provided by the observation that glutamate-induced cortical neuronal damage can be attenuated by 21-aminosteroids (Monyer et al., 1989), which are novel free radical scavengers and lipid peroxidation inhibitors (Hall et al., 1987).

The neuroprotective value of inhibiting various Ca^{2+}-activated catabolic enzymes warrants exploration. Some exogenous inhibitors are available; for example, the protease inhibitor, leupeptin, was found to reduce muscle injury caused by cholinergic overstimulation (Leonard and Salpeter, 1982). Alternatively, it may be possible to enhance the function of endogenous inhibitory factors such as the calpain inhibitor, calpastatin (Melloni and Pontremoli, 1989).

Reducing free radical-induced injury ought to be a straightforward task; quenching of free radicals can be accomplished by either enzymatic or non-enzymatic agents, and indeed such agents have been reported to attenuate hypoxic-ischemic neuronal injury in several paradigms (Siesjö, 1989). Vitamin E pretreatment reduced injury in global ischemia (Yamamoto et al., 1983); and administration of the free radical scavenger enzymes, superoxide dismutase and catalase, reduced infarct volume in focal ischemia (Liu et al., 1989). The 21-aminosteroid, U74006F, improved outcome after transient carotid occlusion in gerbils (Hall et al., 1988). Benefits of free radical scavengers could go beyond reduction of excitotoxic expression, as free radical formation may mediate other forms of neuronal injury, for example glucose deprivation-induced injury of cultured superior cervical ganglion neurons (Saez et al., 1987).

The greater challenge may not be in identifying effective methods for reducing excitotoxicity, but rather selecting the most useful from the available possibilities. Two questions must be answered for each possible method: how well does it work, and what are its associated adverse effects? As Costa and his colleagues have emphasized (Favaron et al., 1988), a generic problem of methods aimed at antagonizing glutamate neurotoxicity antagonism is the danger of interfering with normal excitatory transmission. That problem is potentially greatest with approaches directed at interference with induction or amplification, which are likely to be shared between excitotoxicity and excitatory signaling. Thus, one must hope to achieve a manageable separation between normal and pathological processes, at least over a term of brief therapy. Approaches directed at blocking the subsequent excitotoxic expression might avoid this problem, and might have an advantage when treatment is delayed and intracellular derangements have already occurred. However, the induction stage of excitotoxicity is likely to be the most easily defined; once induction has taken place, events may become increasingly dispersed and difficult to rein in with specific therapies. It may turn out that the best result can be gained with a multi-part approach: interfering with induction as much as side effect tolerance will permit, and then blocking those major components of amplification or expression that can be safely reached.

The induction interference approach presently most advanced in development is the administration of NMDA antagonists, but before clinical efficacy testing can take place, critical safety issues will have to be addressed. Dangers identified with specific NMDA antagonists include excessive stimulation of cerebral metabolic rate (Kurumaji et al., 1989), reversible neuronal vacuolization (Olney et al., 1989), and behavioral disturbances (Koek et al., 1988). In addition, the special role which NMDA receptors are postulated to play in synapse formation, neurotrophism, and synaptic plasticity (Collingridge and Lester, 1989; Lipton and Kater, 1989) may pose important constraints on the use of antogonists that limit dose, duration of therapy, or application to the developing nervous system.

Other therapeutic approaches

The strategy of reducing the intrinsic vulnerability of brain parenchyma to hypoxic-ischemic insult is based on a logical connection between 2 other complementary treatment strategies, each now also gathering momentum.

Strategy 1. Before brain injury occurs: improve blood flow

This is a plausible frontal assault on initial pathophysiology, and the goal of most prior treatment efforts. The approach certainly works in stroke prevention; reduction of arterial disease by controlling hypertension, and the prophylactic use of antiplatelet agents such as aspirin, are proven methods of reducing the incidence of stroke (Grotta, 1987). Whether improving blood flow will also be effective as an intervention after ischemia onset is not yet established. The first attempts to open thrombosed carotid arteries surgically many hours after occlusion were disastrous because the restitution of arterial pressure into the infarcted brain led to dangerous hemorrhages. But more encouraging results have been obtained in recent studies using agents such as tissue plasminogen activator (tPA) to achieve thrombolysis shortly after vessel occlusion (Zivin et al., 1985; Levy et al., 1989). It is possible that such agents will act synergistically with parenchymal approaches (Zivin, 1989), especially to the extent that improved drug access to ischemic tissue can be obtained.

In addition, there has been growing recognition that the activation of phospholipases and resulting phospholipid breakdown triggered by hypoxia-ischemia may have important deleterious effects on subsequent blood flow. In the presence of

oxygen, either during incomplete ischemia or after post-ischemic restitution of blood flow (reperfusion), arachidonic acid is metabolized to eicosanoids (including leukotrienes, prostaglandins E_1, $F_{2\alpha}$, and H_2, and thromboxane A_2) which can potently induce vasoconstriction, brain edema formation, and blood cell aggregation (Bazan, 1989; Hsu et al., 1989). Thus arachidonic acid metabolism may trigger a delayed period of tissue hypoperfusion capable of augmenting the original ischemic insult. Recent studies have suggested that another result of phospholipid breakdown, formation of platelet-activating factor (PAF), may be a particularly important cause of this delayed post-ischemic hypoperfusion (Braquet et al., 1989b). Pharmacological PAF antagonists, which can attenuate delayed post-ischemic hypoperfusion and other PAF-mediated events such as neutrophil chemotaxis and free radical production, show promise as treatments for ischemic insults in brain and other organs (Braquet et al., 1989a).

Yet another method for therapeutically improving post-ischemic cerebral perfusion has been suggested by observations that electrical stimulation of axons in the cerebellar fastigial nucleus increases cortical blood flow but not glucose utilization (Chida et al., 1989). This maneuver can markedly reduce infarct volume after occlusion of the middle cerebral artery in rats (D. Reis, personal communication).

Strategy 2. After brain injury occurs: enhance functional recovery

Once injury has occurred, it may still be possible to exert a favorable influence on the ability of the acutely injured brain to achieve functional recovery. Transient treatment with amphetamine following frontal cortex ablation in rats or cats produces a lasting improvement in beam-walking ability (Feeney et al., 1982; Sutton et al., 1989); conversely, transient treatment with diazepam causes a lasting impairment of recovery of sensory function in lesioned rats (Schallert et al., 1986). The days following injury may be a period of critical regrowth and synaptic plasticity. Moreover, one can hope that progress in neuronal transplantation will make it possible in the long run to restore lost cells to the brain.

Many unanswered questions

Present investigations of the neurobiology of hypoxic neuronal injury are therapeutically encouraging yet emphasize how little is known about the fundamental mechanisms. Prominent among unanswered questions are the following.

What is irreversible neuronal cell injury?

Neuronal degeneration indisputably indicates irreversible injury, and is an unambiguous endpoint suited to experimental investigation. However, the task of identifying hypoxic injury mechanisms would be facilitated by identification of more subtle indices of injury. It is still unclear at what stage specific perturbations—for example, loss of high energy phosphate esters, disturbances of neurotransmitter metabolism, membrane breakdown, mitochondrial failure, or accumulations of intracellular Ca^{2+}—constitute irreversible injury.

Injury might be operationally defined as any abnormality that impairs cell function. Both structural damage and metabolic derangement can cause such impairments, and both can be reversed, at least up to some point of no return that may reflect a complex weighting of multiple pathological events. Exactly where this critical point lies may depend on the availability of therapeutic interventions. As more powerful interventions are developed, it may be possible to restore normal function to cells with higher levels of injury. Ultimately, the barrier limiting the possibility of recovery may be massive structural damage, especially to cell membranes or genes.

Why do NMDA antagonists protect brain better against focal ischemia than against global ischemia?

Neuroprotective benefits of NMDA antagonists are generally accepted for animal models of focal brain ischemia (Albers et al., 1989), but claims of benefits in global ischemia are controversial; in fact, it has been suggested that some early reported successes might be explained by uncontrolled hypothermia (Buchan and Pulsinelli, 1990). Key differences between the nature of focal and global ischemia may account for a greater involvement of NMDA receptor-mediated injury in the former.

A strong candidate for such a difference is the ischemic "penumbra"—the transition zone of incomplete ischemia which surrounds a region of focal ischemia, but which is absent in global ischemia. Positron emission tomography has raised the possibility that this penumbra can expand outward, recruiting brain tissue into the center zone of dense ischemia and resultant infarction (Hakim, 1987). Overstimulation of penumbral NMDA receptors could be a key event in this outward expansion, mediating a self-propagating cycle of ionic shunting, glutamate release, and excitotoxicity. Siesjö and Bengtsson (1989) have speculated that the state of incomplete energy depletion found in the penumbra may specifically favor excitotoxicity, an attractive postulate which dovetails with recent information about the glutamate system. There are several reasons why NMDA receptor-mediated injury might be greater in the penumbra than in the ischemic core (Fig. 1).

Greater glutamate efflux in the penumbra. ATP may be necessary for Ca^{2+}-dependent release of transmitter glutamate (Nicholls, 1989). Although glutamate efflux may also occur by carrier-mediated processes, the preservation of Ca^{2+}-dependent release might enhance net efflux.

Greater NMDA receptor-channel complex phosphorylation in the penumbra. NMDA-induced currents diminish by about half in whole cell recordings unless high-energy phosphates are present in the recording pipette, suggesting that phosphorylation may be needed to maintain the NMDA receptor-channel complex in its most active state (Mody et al., 1988).

A higher pH in the penumbra [about 6.7 compared with about 6.4 in the ischemic core (Hakim, 1987; Siesjö, 1988)]. NMDA receptor-mediated currents in hippocampal neurons are attenuated at the lower end of this pH range (Morad et al., 1988), a finding confirmed in cultures of cortical neurons (Giffard et al., 1990). In cell culture, reducing the pH to 6.4 decreases glutamate neurotoxicity, hypoxia-induced $^{45}Ca^{2+}$ accumulation, and hypoxic neuronal degeneration (Giffard et al., 1990; Tombaugh and Sapolsky, 1990).

Availability of oxygen in the penumbra. Oxygen availability accelerates the formation of superoxide and hydroxyl radicals, and thus might enhance the expression stage of excitotoxic damage. This mechanism is likely to explain the paradoxical finding that incomplete ischemia can be more damaging than complete ischemia (see above).

Thus the conditions found in the penumbra may provide a lethal singularity, at least with regard to NMDA receptor-mediated injury. The level of available metabolic energy may be low enough to trigger excitotoxicity. (Indeed, partial impairment

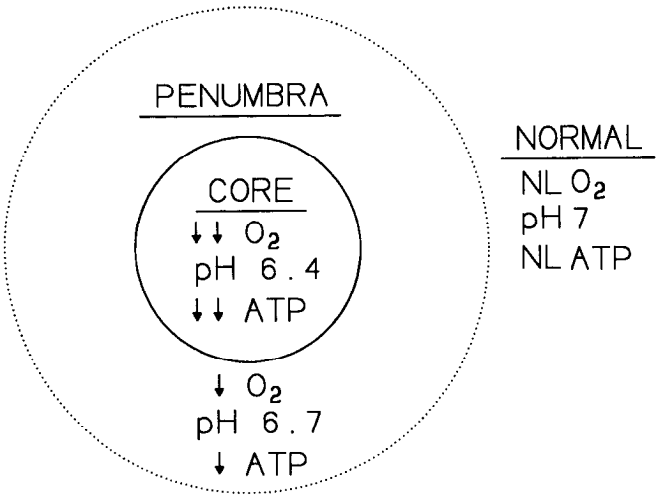

Figure 1. Penumbra: zone of excitotoxicity?

of energy-dependent Ca^{2+} pumps may be necessary for injury to occur.) Yet available energy and pH may be high enough to maintain Ca^{2+}-dependent glutamate release and NMDA receptor function, and enough oxygen may be available to accelerate free radical formation. Some penumbral neurons conceivably might even span different energy zones, such that cell domains in zones with higher levels of energy might help maintain energy-dependent processes in lower energy zones, with deleterious consequences.

In contrast, the conditions of global ischemia may only transit such a critical state, before settling into a state of total energy depletion, low pH, and anoxia, where NMDA receptor-mediated injury is blocked and cells die for other reasons (see below). Perhaps the length of time spent in this transitional state determines the extent to which NMDA receptors may contribute to global ischemic injury. If glutamate is released, but NMDA receptors are inactivated by dephosphorylation or extracellular acidosis, non-NMDA receptors could play a larger role in pathogenesis. It is noteworthy that the new non-NMDA antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) is reported to reduce global ischemic injury in the gerbil (Sheardown et al., 1990).

Although NMDA antagonists may have limited value in the treatment of global ischemia, they are beneficial in the treatment of another global insult—hypoglycemia (Wieloch, 1985). This discrepancy could be explained by the arguments presented above: hypoglycemia produces only incomplete energy depletion, and perhaps, most importantly, it is not associated with acidosis (Auer and Siesjö, 1988).

Why do glia die (and why do NMDA antagonists sometimes prevent this death)?

NMDA receptor-mediated processes may help explain why neurons are so vulnerable to hypoxic injury, but cannot directly explain why glia or endothelial cells, which lack NMDA receptors, also eventually succumb to hypoxic injury. Hypoxia may injure non-neuronal brain cells by mechanisms which qualitatively resemble those underlying glutamate neurotoxicity, but differ quantitatively in how rapidly excessive Ca^{2+} influx can occur without the conduit provided by NMDA receptor-gated channels. Like glutamate neurotoxicity (see above), anoxic injury of isolated rat optic nerves is attenuated by removal of extracellular Ca^{2+} (Stys et al., 1990).

Unexplained, then, is the ability of NMDA antagonists to reduce brain infarction—including non-neuronal cell loss—in the setting of focal brain ischemia. Perhaps the overstimulation of neuronal NMDA receptors somehow facilitates non-neuronal cell death by adversely altering the local environment. This alteration could take the form of changes in ionic concentrations, generation of free radicals or toxic metabolites, or release of catabolic enzymes. As outlined above, phospholipases activated by NMDA receptor-mediated Ca^{2+} influx could enhance the formation of eicosanoids and PAF, leading to increased tissue ischemia.

A particularly important alteration may be extracellular lactic acidosis, which may be promoted by NMDA receptor-induced mitochondrial failure and energy depletion. Lactate acidosis has been proposed as the direct cause of glia injury in infarction (Plum, 1983). Glia are more vulnerable to acid-induced injury than neurons (Goldman et al., 1989); Norenberg et al. (1987) found that 1–2 hr of exposure to pH 6 caused chromatin clumping and mitochondrial swelling. We have found that a pH of 6.4 for 8 hr kills large numbers of cultured cortical glia (R. Giffard, H. Monyer, and D. Choi, unpublished observations). Thus despite producing a beneficial reduction of NMDA receptor-mediated neuronal loss, ischemic acidosis might potentiate glial damage and foster infarction.

Is reduction of neuronal death always desirable?

While neuronal death is a convenient endpoint for the assessment of new treatment strategies, the ultimate goal of treatment is functional benefit. Improved neuronal survival need not always translate into functional benefit—for example, if a treatment improved the survival of damaged neurons, led to the formation of incorrect synaptic connections, or disturbed critical synaptic plasticity following injury.

Of all the problems that might theoretically arise with the therapeutic use of NMDA antagonist in cerebral ischemia, the possibility of interference with functional recovery is probably the most worrisome. Most other anticipated side effects are likely to be tolerable, especially if treatment duration can be brief and hospital life support facilities are available. Demonstration of functional benefit is a mandatory experimental hurdle for any new treatment strategy.

What is the predictive value of current experimental models of cerebral ischemia?

The development of stroke treatments is progressing apace, but has not yet resulted in a proven clinical success. Until such success has been attained, the predictive value of specific experimental models will remain unknown. While the development of clinical therapy would be much enhanced by the availability of experimental models capable of accurately predicting treatment efficacy in patients, such perfect models may never be found.

In vitro model systems for the study of hypoxia obviously lack many features relevant to stroke *in vivo*. Moreover, methods for inducing ischemia in animals may introduce substantial perturbations that do not reflect the pathophysiology of human stroke (Wiebers et al., 1990); and extensive experience with rodent models has emphasized the importance of differences in species or experimental technique in determining outcome (Ginsberg and Busto, 1989). The future success of stroke re-

search will probably depend on understanding basic principles well enough to allow extraction of meaningful information even from flawed models. Clinical trials may ultimately have to be designed—cautiously—on the basis of our best extrapolations from such information.

Closing comments

Perhaps the most important lesson provided by the last few years of stroke research is an alteration of scope. Previously, relatively few investigators considered the neurobiology of hypoxia-ischemia a promising area for study. It was widely assumed that neuronal damage occurring in ischemia was a direct—and hence inevitable—consequence of metabolic supply falling below tissue demand. This "brain as an organ" concept was not one that fired imaginations; as a result, the creative search for therapy centered on improving blood flow. Regardless of the specific fate of approaches under current consideration, it seems safe to predict that recent progress toward understanding the cellular nature of hypoxic neuronal injury has permanently expanded the traditionally narrow boundaries of stroke research.

In conformation with many precedents in scientific progress, the archetypically applied field of stroke research is now benefiting from much prior work in basic neuroscience. The chemistry and physiology of synaptic transmission and the intricate weft of its underlying molecular events may prove to be astonishingly relevant to understanding why neurons die after exposure to hypoxia. Stroke research shows promise of becoming a bridge between the clinical and basic neurosciences, an arena where clinicians and scientists trained in different disciplines may profitably interact. We can hope that such interactions will catalyze the insights necessary to develop effective therapies for stroke—and ultimately, effective therapies for other diseases of the nervous system.

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