Cellular defenses against excitotoxic insults

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Abstract

The cellular events mediating necrotic neuron death are now reasonably well understood, and involve excessive extracellular accumulation of glutamate and free cytosolic calcium. When such necrotic neurological insults occur, neurons are not passively buffeted, but instead mobilize a variety of defenses in an attempt to decrease the likelihood of neuron death, or to decrease the harm to neighboring neurons (by decreasing the likelihood of inflammation). This review considers some of these defenses, organizing them along the lines of those which decrease neuronal excitability, decrease extracellular glutamate accumulation, decrease cytosolic calcium mobilization, decrease calcium-dependent degenerative events, enhance neuronal energetics, and bias a neuron towards apoptotic, rather than necrotic, death. Although these are currently perceived as a disparate array of cellular adaptations, some experimental approaches are suggested that may help form a more unified subdiscipline of cellular defenses against neurological insults. Such an advance would help pave the way for the rational design of therapeutic interventions against necrotic insults.

Keywords: excitotoxicity, heat shock proteins, necrotic neuron death, oxygen radicals, retaliatory neurotransmitters.


Few arenas of disease can be as devastating as the damage to the nervous system following excitotoxic insults such as the global ischemia of cardiac arrest, the focal ischemia of a stroke or a major-seizure disorder. For these reasons, tremendous amounts of research have focused on the mechanisms underlying excitotoxic neuron death, and out of this has come a cascade that is likely to be familiar to most readers. Such insults involve synaptic accumulation of excessive glutamate, which, at sufficiently high levels, becomes neurotoxic. This is primarily mediated by the mobilization of free cytosolic calcium in the postsynaptic neuron. This, in turn, leads to the generation of oxygen radicals, energetic collapse, damage to the cytoskeleton, protein misfolding and, in a subset of neurons, the triggering of apoptosis (Lee et al. 1999).

This glutamatergic cascade of cell death now approximates something resembling a central dogma in neurology, and research ranging from pharmacology to gene therapy aims to protect neurons by targeting critical steps. As the central point of this review, neurons are not passively buffeted by glutamate, calcium and oxygen radicals. Instead, various adaptations can be mobilized by neurons (and, in some cases, by neighboring glia), which should be thought of as constituting neuronal defenses. ‘Defenses’ are defined, in this case, as processes that are either uniquely activated in response to injury, or are activated above basal levels in response to injury, and that decrease the likelihood of neurotoxicity occurring. Some examples are so central to the cell biology of neurons that it becomes difficult to perceive them to be defenses. For example, the low-affinity/high-capacity sequestering of calcium by mitochondria in response to elevated cytosolic calcium concentrations can be viewed as a neuronal defense. In contrast, a variety of these defenses are likely to be less familiar to readers.

This review considers some of the defenses available to neurons (or those activated by glia in defense of neurons). While some of these have been uncovered only recently, many have been known for some time. In broadly reviewing the range of such defenses (the first such review, to my knowledge), the goal is to begin to discern some of the common themes underlying their activation. This is a first step in organizing these disparate phenomena into a unified subject of neuronal defense systems. As will be discussed,
such a unification is likely to facilitate the development of certain therapeutic interventions in clinical neurology.

Examples are organized on a time scale, moving from the earliest to the final events of excitotoxic injury; in the interest of brevity, neither are all of the categories of defense, nor are all the examples within each category, included.

Defenses that decrease neuronal excitability

The opening of potassium channels at the peak of action potentials limits and rectifies neuronal excitability; this is basic to any introductory neuroscience course. Amid the vast heterogeneity of potassium channels, a few can be specifically activated by insults, and the proximal mechanisms triggering activation establish themes to be echoed throughout this review.

A first example would be the small-conductance (SK) calcium-dependent potassium channel (Sah 1996). While various potassium channels show some degree of calcium sensitivity, SK channels are highly calcium-dependent and are quite sensitive to transient excesses of cytosolic calcium, opening at submicromolar concentrations (Blatz and Magleby 1987). Such channels are ideally suited to transduce the calcium mobilization central to excitotoxic injury into a protective, hyperpolarizing signal. Commensurate with that, SK channels mediate the after-hyperpolarization (AHP) that causes the refractory period (Madison and Nicholl 1984; Lancaster and Adams 1986; Sah 1996), and potentiation of the AHP by excessive cytosolic calcium would certainly dampen the excitability. Thus, SK channels play a key role in spike-frequency adaptation (when a train of action potentials results in a compensatory and protective lengthening of the AHP; Madison and Nicholl 1984; Sah 1996).

As evidence of the protective potential of SK channels, the selective blockade of which can have neurodegenerative consequences (Mourre et al. 1989).

A second example is the ATP-dependent potassium channel (K-ATP), the opening of which is triggered by ATP depletion (Heurteaux et al. 1993). Regulation by ATP is ideal for transducing the energy depletion characteristic of excitotoxic insults into a protective response. Originally characterized in the pancreas, where it is involved in insulin secretion, the neurobiological relevance of K-ATP was initially thought to be restricted to brainstem and hypothalamic glucoreceptor neurons (Spanswick et al. 1997; Silver and Erecinska 1998). It is now thought that the K-ATP channel also plays a role in the sequelae of excitotoxic injury in the hippocampus. As evidence, its conductance is enhanced in the hippocampus by energy depletion (Fujiwara et al. 1987; Politi and Rogawaski 1991; Riepe et al. 1992) and by excitotoxic insults, which indirectly disrupt energy stores (Trapp and Ballanyi 1995). At presynaptic sites, such as in the CA3 region of the hippocampus, such activation inhibits glutamate release. These should constitute protective effects and, in support of this, K-ATP channel activation blunts anoxic depolarization in hippocampal slices (Ben-Ari 1990), protects neurons in various brain regions from glutamatergic insults (Heurteaux et al. 1993; Lauritzen et al. 1997; Wind et al. 1997), and has been implicated in ischemic preconditioning (Heurteaux et al. 1995).

An additional neuronal defense is to decrease excitability by stabilizing the resting potential. In cultured cerebellar neurons, NMDA-mediated calcium influx, via binding to calcineurin, dephosphorylates the Na+/K+/ATPase and increases its activity (Marcaida et al. 1996).

Thus, two reliable consequences of excitotoxic insults (the mobilization of cytosolic calcium and the depletion of ATP) serve to either stabilize the resting potential, or to protectively activate potassium channels. Collectively, these would be likely to decrease neuronal excitability in the face of an excitotoxic challenge.

Defenses that decrease glutamate accumulation in the synapse

Given the pivotal role of glutamate in excitotoxic injury, adaptations that act to decrease synaptic accumulation of glutamate can potentially be markedly protective. As discussed in the last section, the activation of presynaptic K-ATP channels can inhibit glutamate release (secondary to decreased excitability). A number of additional protective mechanisms exist that inhibit such release during insults. A number of well-characterized ones involve retrograde signaling of inhibitory neurotransmitters.

A first and well-known ‘retaliatory’ system involves the inhibitory neurotransmitter GABA (Bradford 1995). The retrograde signalling of GABA is multisynaptic, in that collaterals from neurons (e.g. glutamatergic pyramidal neurons in the hippocampus) terminate on GABAergic interneurons which, in turn, inhibit glutamatergic neurons. The importance of this protective feedback loop is demonstrated by the epileptogenic effects of GABA-receptor antagonists, the clinical use of GABA agonists to control seizures, and the evidence that a failure of this GABAergic pathway may underlie some forms of epilepsy (During et al. 1995).

Related to this is the release of taurine during insults (Magnusson et al. 1991; Torp et al. 1991). The inhibitory amino acid is contained in glial cells and is released during glutamatergic excess. This is primarily thought to occur as a result of the uptake of potassium (and water) by glia, causing swelling and stretch-activated taurine release (Martin et al. 1990). Taurine decreases presynaptic neuronal excitability by increasing chloride influx (Huxtable 1992), and recent evidence suggests that this results from binding to the GABA-A receptor (O’Byrne and Tiptin 2000). The net result is neuroprotective actions similar to those of GABA itself (O’Byrne and Tiptin, 2000).
The release of adenosine constitutes an additional
retaliatory system (first presented as a hypothesis by
Dragunow and Faull 1988). The accumulation and release
of adenosine is a logical index of a state of severe energy
depletion, in that such release requires the successive
depletion of ATP, ADP and then the final dephosphorylation
of AMP, to form adenosine (Latinì et al. 1996). Such
adenosine has effects on cerebral vasculature that can be
construed as a defense, and will be considered below. Of
relevance to this section, adenosine released from post-
synaptic spines travels in a retrograde manner to inhibit
presynaptic glutamate release. This is accomplished through
binding to A1 adenosine receptors linked by G proteins to
both calcium and potassium channels.

As would be predicted, adenosine and adenosine agonists
have considerable neuroprotective potential (Turski et al.
1985; Evans et al. 1987; Daval et al. 1991; Dux et al. 1992;
Mori et al. 1992). Conversely, pharmacological antagonism
of adenosine receptors worsens necrotic damage and
prolongs seizures (Thurston et al. 1978; Turski et al.
1985; Dragunow and Robertson 1987; Lekieffre et al. 1991;
Calabresi et al. 1997). Moreover, it has been suggested that
adenosine is a natural anticonvulsant, and that failure of this
adenosine defensive mechanism underlies the prolonged
seizures that characterize status epilepticus (Young and
Dragunow 1994). In addition, some circumstances of
neuroprotection mediated by exogenous adenosine appear
to be receptor-independent, and this has been explained with
the eminently logical observation that insofar as adenosine
can be the end product of ATP hydrolysis, it can be a
precursor for ATP resynthesis as well (Jurkowicz et al.
1998).

A number of compensations may regulate extracellular
 glutamate concentrations by altering glutamate uptake from
the synapse; of relevance to understanding this literature, it
should be noted that such uptake is brought about by both
neurons and glia, with the latter serving the far more
important role. Ischemia can rapidly up-regulate glutamate
transport, and does so preferentially in the hippocampus and
cortex (Anderson et al. 1993). The proximal mechanism
underlying this phenomenon is not clear, and it is also not
clear the extent to which this is a neuronal and/or glial
effect. Initially, this is readily interpreted as protective,
insomuch as this would facilitate glutamate removal from the
synapse. However, the collapse of ionic gradients during
severe necrotic insults can cause the reversal of transporters,
resulting in the export of plentiful amounts of glutamate
from the cytosol of both the presynaptic neuron and glia
(Rossi et al. 2000). Because of this, increased numbers of
transport sites will be protective only if restricted to
postschismic periods when gradients have been restored.
An additional cellular response to ischemia is relevant to
this issue. Hypoxic-ischemic excitotoxicity leads to intra-
cellular acidosis (caused by the reliance upon anaerobic
metabolism and the production of lactic acid). Such acidity
can inhibit the activity of the glial glutamate uptake pump
(at least in salamander retinal glia); this is accomplished by
markedly decreasing the affinity of the carrier for sodium,
which is cotransported with glutamate. In the early stages of
ischemia this is likely to impair glutamate removal from the
synapse, and most definitely is not a protective defense.
However, the majority of the ischemic period is spent with
the pump reversed, thereby exporting glutamate. At such
times, the ability of acidosis to inhibit the activity of the
glutamate pump has been interpreted as being protective
(Billups and Attwell 1996).

Thus, a number of mechanisms exist, as direct or
indirect consequences of sustained excitation, for delimiting
glutamatergic signaling.

Defenses that limit calcium mobilization in the
postsynaptic neuron

Varied routes exist for calcium mobilization during insults,
including influx via NMDA-receptor-gated and voltage-
gated channels, some degree of influx via AMP-A receptors,
and release from intracellular stores. A number of the
downstream consequences of such calcium mobilization act
as signals to inhibit subsequent mobilization. Some may
decrease the number or efficacy of glutamate receptors,
while others alter the extent of calcium mobilized in
response to glutamate receptor activation.

An example of defenses that decrease glutamate receptor
number involves calcium-mediated activation of calpain.
This protease has gained attention in the neuron-death
literature, in that its activation can lead to proteolytic
cleavage of cytoskeletal proteins such as spectrin (Seubert
et al. 1988). This is thought to help bring about the collapse
of the cell body (i.e. pyknosis) that characterizes the
necrosis seen after numerous insults. As a defense, however,
such calpain activation can also result in proteolysis of
both NMDA and AMP-A receptors, thereby decreasing
the vulnerability to glutamate (Bi et al. 1996, 1998a,b).
This constitutes the mechanism that endocrinologists
refer to as an ‘ultra-short’ feedback loop, in which the
most proximal consequence of a biological process subse-
quently inhibits that same process. As a more distal
example, the caspases activated as part of apoptotic
pathways in response to necrotic injury can also lead to
the cleavage of glutamate receptors. This will be reviewed
later, at length.

A number of defenses decrease the extent of calcium
mobilization in response to glutamate, each forming a
feedback loop. As the most proximal example, calcium itself
can mediate such a negative feedback role. Calcium-
dependent activation of calcineurin and calmodulin can
inhibit voltage-gated and NMDA-receptor-gated calcium
currents, respectively (Vyklicky 1993; Lieberman and Mody
A less proximal feedback loop involves the ischemia-induced acidity that was just discussed. Such protons can inhibit NMDA-receptor activation (Tang et al. 1990; Traynelis and Cull-Candy 1990; Takadera et al. 1992); as such, moderate acidosis can actually be neuroprotective via this route (Giffard et al. 1990; Tombaugh and Sapolsky 1990). This has helped overturn an older view that acidosis is uniformly damaging to neurons and is the central mechanism by which selective neuron loss occurs during hypoxia-ischemia (Tombaugh and Sapolsky 1993).

Another downstream consequence of necrotic insults, as discussed, includes the energy depletion that gives rise to adenosine generation. In addition to its protective pre-synaptic effects, adenosine decreases calcium currents in response to glutamate (Phillis and Wu 1981), forming another feedback loop.

As the most distal example, a key downstream consequence of glutamatergic excitotoxicity is the generation of oxygen radicals. This includes nitric oxide, which can diffuse to neighboring neurons, generating oxidative damage. However, nitric oxide also acts intracellularly to nitrosylate the NMDA receptor, inhibiting its activity (Lipton et al. 1993). This may explain the long-standing observation that neurons containing NADPH diaphorase (a dated term for nitric oxide synthase) are relatively resistant to excitotoxic injury, as compared with (neighboring) neurons lacking the enzyme.

Thus, a number of routes exist whereby calcium mobilization into the cytosolic compartment inhibits subsequent mobilization, via something metaphorically akin to the end-product inhibition of classical biochemistry.

Defenses that protect against calcium-dependent degenerative effects

As noted, calcium excess ultimately leads to the generation of oxygen radicals, cytoskeletal degradation and the misfolding of proteins. A number of adaptations delimit some of these adverse consequences. A well-characterized one concerns protein misfolding. It remains unclear whether the damage that arises through this route is solely a result of the loss of function of proteins (when they misfold), or whether there is ‘gain-of-function’ damage caused by the aggregation of such proteins (as is becoming a dominant theme in some slowly emerging neurodegenerative disorders, which are coming to be known as ‘conformational’ diseases). In either case, a consistent response of neurons (or any cell) to cellular stress is the induction of heat-shock proteins (hsp), which protect against such misfolding. An enormous number of early studies demonstrated that hsp expression correlated with resistance to necrotic injury (reviewed in Yenari et al. 1999). More recent studies have suggested a causal role for hsp expression in this correlation, in that the overexpression of hsp can protect against the toxicity of necrotic insults (Yenari et al. 1998).

Another relevant defense is the up-regulation of antioxidant capabilities following necrotic insults. This includes the increased activity of Mn- and CuZn-superoxide dismutase (SOD) (Ohtsuki et al. 1993; Fukuhara et al. 1994; Matsuyama et al. 1994; Bruce et al. 1996; Mattson et al. 1997; Toyoda et al. 1997; McIntosh et al. 1998), glutathione peroxidase and catalase (cf. Azbill et al. 1997; Goss et al. 1997; McIntosh et al. 1998). The proximal signal for such up-regulation is unclear in most cases, as is whether each instance involves a transcriptional increase in the synthesis of a new enzyme, the inhibition of degradation or the increased activity of a pre-existing enzyme. One exception concerns the insult-induced up-regulation of Mn-SOD secondary to the activation of NFκB. Normally, this transcription factor is inactive, thanks to the inhibitory actions of IκB. However, excitotoxic insults can lead to the inactivation and degradation of IκB; the calcium-mediated phosphorylation of which leads to its proteosomal degradation, while there is evidence that oxidative damage to IκB can inactivate it as well. Once disinhibited, NFκB increases the transcription of Mn-SOD among a number of other potentially protective genes (see below; reviewed in Mattson et al. 2000) [It should be noted, amid this picture of the protective effects of NFκB, that the activation of NFκB in some models of insults appears to mediate, rather than decrease damage (Schneider et al. 1999; Nakai et al. 2000)]. Regardless of the mechanisms by which antioxidant enzyme activity increases posts insult, such increased activity is likely to be neuroprotective, given the salutary effects of experimental overexpression of such enzymes during excitotoxic insults (Kindy et al. 1996; McIntosh et al. 2000).

During such insults, there is also likely to be an export of ascorbic acid from astrocytes to neurons. One report demonstrates this as a direct consequence of the glial stretching, which is a consequence of the osmotic changes in glia caused by their uptake of potassium (Siushansian et al. 1996). There also exists an ascorbate/glutamate antiporter in the brain, driven by glutamate uptake rather than by glutamate binding to its receptors (Cammack et al. 1991; Miele et al. 1994). Such an ascorbate/glutamate antiporter appears to occur in both neurons and glia. This efflux in response to glutamate excess has typically been interpreted as a maladaptive loss of antioxidant potential during insults (Rice 2000). However, given that the vast majority of glutamate uptake from the synapse is via glia, this is likely to be a mechanism for the transfer of ascorbate from astrocytes to neurons during insults. Should this indeed be occurring, this is likely to be protective given the beneficial effects of ascorbate supplementation against necrotic insults both in vitro and in vivo (Sciamanna and Lee 1993; Atlante et al. 1997; Henry and Chandry 1998; Brahma et al. 2000). It remains unclear whether these protective actions are entirely attributable to ascorbate acting as an antioxidant.
Defenses that enhance neuronal energetics

A key to understanding excitotoxic insults is appreciating that they are ultimately energy crises, either resulting from impaired generation of energy (as in hypoxia-ischemia or hypoglycemia), or pathologically elevated energy demands (as in a sustained seizure). Some protective responses target the energetic vulnerability. For example, ischemia up-regulates the expression of glucose transporters both in vivo and in vitro (Lee and Bondy 1993; Gerhart et al. 1994; Klip et al. 1995, 1996; McCall et al. 1996; Urabe et al. 1996). This involves increased expression of both the Glut-1 transporter, which is predominately found in microvessels and glia, as well as Glut-3, which is preferentially located in neurons.

In general, the increase of Glut-1 levels is more rapid and more dramatic than that of Glut-3 levels. Some of the rise in Glut-1 levels probably reflects the microglial proliferation that follows injury, as the increase is accompanied by an increase in GFAP. However, there is also likely to be increased expression per glial cell, and the increase localized to microvessels is likely to represent a true increase in amount of transporter mRNA per cell as well. As a related issue, the increase in Glut-3 typically seen 24 h post-ischemia is usually transient, and the later decline is likely to reflect the dying of neurons themselves (Vannucci et al. 1996). As a final consideration, the caution that must always be exercised in extrapolating from a change in the level of message to that of functional protein itself is particularly critical here, given the decreased levels of protein synthesis that are a hallmark of the postischemic brain tissue. It is likely that translation of message into glucose transporter is somewhat spared from this inhibition of protein synthesis, given that glucose transporters are regulated in a tightly coordinate fashion with GRP-78 stress proteins (Wertheimer et al. 1991). This is probably the case in less severely injured tissue. Thus, in transient focal ischemia, while the increased levels of transporter message are not translated into increased levels of protein in the ischemic core, there is a coordinate increase in protein levels in the penumbra (Urabe et al. 1996).

An increase in the levels of glucose transporter and glucose transport is certainly likely to be protective in brain tissue following an insult, given the well-characterized dependence of neurons on glucose. However, this dogma regarding neuronal dependence has recently undergone some revision, with a recognition of the importance of lactate (Magistretti et al. 1999). Glia with endfeet bordering capillaries can take up glucose from the circulation and convert it to lactate for export to neurons. This occurs with no change in oxygen content, which indicates that this is ‘intentional’ lactate generation rather than being secondary to anaerobic metabolism (Hu and Wilson 1997). Neurons not only utilize such lactate, but also recover more effectively from insults when utilizing lactate, rather than glucose (Schurr et al. 1998). This is probably related to it requiring fewer biochemical steps from lactate to oxidative phosphorylation than from glucose, plus the fact that glucose, unlike lactate, requires an initial investment of ATP in order to yield energy.

There are a number of adaptations in this multicellular metabolic unit that enhance lactate delivery to neurons during insults. At the systemic level, sympathetic-nervous-system activation and secretion of glucocorticoids mobilize glucose into the circulation. This is predominately accomplished through glycogenolysis throughout the body and gluconeogenesis in the liver (Sapolsky et al. 2000). At the vascular level, there is typically an increased perfusion rate and the recruitment of additional capillaries in response to excitotoxic injuries, as well as a loosening of the blood–brain barrier, thereby enhancing glucose availability. Mediators of this include adenosine binding to A2 adenosine receptors on vasculature, and the induction of endothelial NOS (Erecinska and Silver 1994; Samdani et al. 1997; Sweeney 1997). Next, there is stimulation of glial uptake of glucose, and of glial glycogenolysis, resulting in the generation and release of lactate (Magistretti et al. 1986, 1999; Sonnewald et al. 1997; Wiesinger et al. 1997). Proximal mechanisms for this include elevated synaptic concentrations of glutamate and arachidonic acid, both of which stimulate enhanced glucose uptake (Yu et al. 1993), and of adenosine, which stimulates glial glycogenolysis (Magistretti et al. 1986). In addition, the increased levels of glucose transporter, as just described, would contribute to this glial effect. Finally, there is a shift towards preferred neuronal uptake and/or metabolism of lactate over glucose, brought about by both adenosine and the presence of high concentrations of lactate itself (Bliss and Sapolsky 2001).

An additional metabolic adaptation concerns mitochondria. As noted, such organelles serve as high-capacity sinks for cytosolic calcium during insults. Thus, the uptake of large amounts of calcium into the mitochondrial matrix logically signals neuronal distress. Commensurate with this, such uptake potently stimulates enzymes of oxidative phosphorylation (McCormack and Denton 1993).

Apoptosis and the inhibition of apoptosis as possible defenses

A decade ago, the study of apoptotic, programmed cell death appeared to be of relevance to understanding excitotoxic insults only insofar as it taught us which mechanisms were irrelevant to neuron death occurring after such injury. The more recent recognition that either classical and complete apoptosis, or at least elements of it, occur in a subset of excitotoxically injured neurons has spurred a tremendous amount of research. Two general ideas have emerged regarding the logic of such apoptosis. The first is that in instances where a neuron is irreversibly moribund following...
an insult, apoptotic death is preferable to necrosis. This is because the former minimizes the inflammation that can be a major source of secondary neuronal injury (Raff 1998; Nicotera et al. 1999). The second idea concerning apoptosis is that because such programmed cell death is a costly, active process, energy availability is a major determinant of whether apoptosis is initiated, and whether it is carried out to completion in a dying neuron. Intrinsic in this idea is the explanation of why only a subset of neurons show apoptotic markers postinsult (Ankarcrona et al. 1995; Eguchi et al. 1997; Leist et al. 1997; Roy and Sapolsky 1999).

A number of papers have reported up-regulation of the anti-apoptotic protein bcl-2 in brain tissue following excitotoxic insults (e.g. Clark et al. 1999), or the preferential up-regulation in neurons with a greater likelihood of surviving (for example, in the ischemic penumbra; Isenmann et al. 1998). As one possible mechanism for this, bcl-2-family proteins are under the transcriptional control of NFκB (Mattson et al. 2000). Does such up-regulation constitute a protective defense? In theory, this may not be the case, in that inhibition of apoptosis (in this case, by inhibition of caspases) may only result in a default to a neuron instead of dying necrotically (Lamaire et al. 1998). Insofar as this would increase inflammation, this would exacerbate damage rather than be protective. Despite that, a number of investigators have overexpressed bcl-2 therapeutically following insults, reporting that it decreases the overall extent of damage, rather than merely shifting from apoptotic to necrotic cell death (Linnik et al. 1995; Jia et al. 1996; Lawrence et al. 1996; Antonawich

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**Fig. 1** Defenses mediated most proximally by the rise in free cytosolic calcium concentrations. (1) The opening of calcium-dependent potassium channels. (2) Increased activity of the Na⁺/K⁺/ATPase, caused by its calcium-dependent dephosphorylation. (3) This route encompasses a number of separate effects, namely the degradation of glutamate receptors, as a secondary consequence of the calcium-dependent activation of calpain, and the inhibition of both receptor-gated and voltage-gated calcium currents, as a secondary consequence of the calcium-dependent activation of calmodulin and calcineurin. (4) The calcium-dependent enhancement of oxidative phosphorylation.

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**Fig. 2** Defenses mediated by the energy depletion intrinsic to excitotoxic challenges. (1) The opening of ATP-regulated potassium channels. (2) Adenosine-mediated inhibition of glutamate release. (3) Adenosine-mediated cerebrovascular changes to enhance glucose uptake. (4) A shift towards neuronal uptake and/or metabolism of lactate in preference to glucose, triggered by adenosine.
et al. 1999). This seeming paradox is explained by the increasing appreciation that bcl-2 does not merely antagonize the actions of the pro-apoptotic protein BAX (thereby preventing apoptosis), but stabilizes the mitochondria as well (thereby also protecting against necrosis; reviewed in Green and Reed 1998; Vander Heiden and Thompson 1999).

Therefore, up-regulation of bcl-2 following an insult should be viewed as protective only insofar as the main consequence is to decrease the overall extent of neuron death, rather than shifting death from apoptosis to necrosis.

A key event in apoptosis, downstream of BAX activation, is the activation of the proteolytic cascade of caspases. A recent report concerns an unexpected consequence of caspase activation, namely the proteolytic degradation of AMP-A receptors. As a result, this decreased neuronal vulnerability to excitotoxin-induced necrosis (Glazner et al. 2000). This can be interpreted as a protective response along the lines of the logic in the preceding paragraph – once there is the certainty of neuron death, a shift from necrosis to apoptosis could be viewed as protective.

Considerations and conclusions
This only represents a partial review of defenses against insults. Subjects omitted include the potentially protective roles of metabotropic glutamate receptors, presynaptic glutamate autoreceptors (e.g. Scanziani et al. 1997), the induction of CREB (Walton and Dragunow 2000) and the protective effects of endogenous neurotrophins (Mattson et al. 1993; but also see Gwag et al. 1995 for evidence of neurotrophins worsening insults).

Amid the numerous defenses discussed (a schematic representation of an array of defenses against necrotic...
insults are summarized in Figs 1–4), a theme that emerges is that the dominant mediators of damage are the most proximal triggers of defenses. Thus, the accumulation of extracellular glutamate activates the lactate pathway of energetic defense; the mobilization of cytosolic calcium triggers an array of the defenses; the accumulation of protons or nitric oxide decrease the excitability of the NMDA receptor; ATP depletion leads to adenosine release and activation of K-ATP channels. These are all logical feedback and feed-forward pathways. If the endangering Signal X is detected, it is adaptive that this should serve as the trigger to inhibit further generation of Signal X, as well as to defend against the downstream consequences of Signal X.

Despite the collective logic of these defenses, they are nevertheless a heterogeneous array of processes, involving intra- and intercellular actions, adaptations in neurons, glia, vasculature and peripheral organs have been documented to occur to differing extents in an array of different brain regions and in response to differing insults. Also, importantly, the study of such defenses represents an unsystematic ‘hodge-podge’ for the very reason that this is not yet a coherent field in neuroscience. To my knowledge, this review is one of the few, if not sole, attempts to organize some of these cellular responses under the rubric of a defense system.

A first step towards forming this into a coherent field would be for the study of a multitude of these defenses within the same cells, in the same experimental preparation, and with identical insults. This would allow a number of key questions to be answered.

(i) For a particular insult and brain region, what is the hierarchy of the activation of these defenses? A temporal version of this question would be ‘Which defenses are activated most rapidly after the onset of an insult, and which are activated last?’ or, ‘Which defenses are activated by the mildest version of the insult, and which have the highest threshold, being activated by only the most severe insult?’.

(ii) For a particular insult and brain region, when does the mobilization of a defense saturate? In other words, at what point in time after an insult, and at what severity of an insult has a defense reached its maximal efficacy?

(iii) For a particular insult and brain region, when does the mobilization of a defense begin to abate?

(iv) For a particular insult and brain region, how protective is a particular defense – does this represent a potently protective intervention, or one more akin to sponging up the ocean?

Studies such as these would be of heuristic value in understanding the ‘priorities’ of an endangered neuron. They will also allow for the design of rational therapeutic interventions. For example, it will make sense to focus efforts on the pharmacological activation of a particular defense if it has been shown to be a particularly powerful one, or one which, while potentially protective, subsides rapidly posts insult. Conversely, it would make little sense to pharmacologically activate a particular defense if its endogenous version has already saturated its protective potential. By understanding both the ‘wisdom’ of the nervous system and the limits it faces in defending itself, efficacious clinical therapies may well emerge.

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References


