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CHAPTER 2

Glucocorticoids, hippocampal damage and the glutamatergic synapse

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Introduction

Organisms are often subject to stressors which disrupt homeostasis; such perturbations produce a set of endocrine and neural adaptations that form the stress-response. Central to this is the secretion of glucocorticoids (GCs) by the adrenal cortex. These steroid hormones are secreted as the final step in an endocrine cascade beginning with the perception of stress in the brain. The hypothalamus, within seconds, secretes corticotropin releasing factor (CRF) along with a number of other minor secretagogues which augment CRF action. These collectively stimulate the pituitary release of ACTH, within approximately 15 sec. ACTH, in turn, stimulates the adrenal secretion of GCs within minutes. The GCs divert energy to muscles by blocking glucose utilization in other tissues and promoting the breakdown of stored energy; they increase blood pressure in synergy with catecholamines. Finally, they suppress costly anabolic processes such as growth, reproduction, and the immune and inflammatory responses until more auspicious times. These GC actions are highly catabolic, underscoring the dramatic responses needed in adapting to stress. Thus when exposure to GCs is prolonged (due to stress, pathologic hypersecretion, or exogenous administration), the deleterious consequences can include myopathy, steroid diabetes, hypertension, impotency, amenorrhea and immunosuppression (Krieger, 1982; Munck et al., 1984).

It has become clear that the deleterious consequences of GC overexposure can also include damage to neurons of the hippocampus. The structure is a principal neural target site for GCs. It (and the septum) are the only regions with appreciable concentrations of the type I corticosteroid receptor, and it has concentrations of the type II receptor equal to any other brain region (Reul and De Kloet, 1985). Moreover, hippocampal neurochemistry and electrophysiology, and hippocampal-dependent behavior are very sensitive to GCs (Meyer, 1985; McEwen et al., 1986). This paper will review the current knowledge concerning the cell biology of how GCs damage hippocampal neurons.

The first evidence of this neurotoxicity was that pharmacologic concentrations of GCs preferentially damage the guinea pig hippocampus (Aus der Muhlen and Ockenfels, 1979). This observation was difficult to interpret at the time, as it predated the first demonstration of corticosteroid receptors in the brain, at which time GCs and the hippocampus were linked together. A decade later, GCs were implicated in playing a physiological role in hippocampal aging in the rat. The first evidence was correlative. Aged male rats typically have elevated basal GC concentrations (reviewed in Sapolsky et al., 1987), and it was shown that the more severe the GC hypersecretion in aged rats, the more pronounced the hippocampal degeneration (Landfield

et al., 1978). Subsequently, the same authors showed that removal of GCs (via adrenalectomy) at mid-age (12 months) prevented the hippocampal neuron loss and cognitive impairments typical of old age in the rat (Landfield et al., 1981). Thus, some adrenal-derived hormone caused hippocampal neuronal aging. We strengthened the case for the hormone being GCs, showing that prolonged exposure to GC concentrations in the upper physiologic range (equivalent to those of major stressors) accelerates hippocampal neuron loss (Sapolsky et al., 1985). The pattern of degeneration resembled that seen in the aged hippocampus: pyramidal neurons of Ammon's horn with high concentrations of corticosteroid receptors were preferentially lost. Moreover, this was accompanied by glial proliferation and infiltration, classic neuropathologic markers of neuronal degeneration. It was then demonstrated that prolonged stress itself could also damage the hippocampus (Kerr et al., 1986).

Research then focused on the mechanisms of GC hippocampal neurotoxicity. It was reasoned that GCs need not be directly toxic but need only be as catabolic as in many peripheral tissues. GCs may, in effect, leave the neurons on the "edge" of a metabolic cliff. With no further challenge, the period of GC exposure passes uneventfully. However, a co-incident challenge might be less readily survived. This idea predicted that varied neurological insults that damage the hippocampus should become more toxic in the presence of more GCs (Sapolsky, 1985a). Work by ourselves and others supports this idea. Insults as varied as excitotoxic seizures due to kainic acid (Sapolsky, 1985a, 1986a; Theoret et al., 1985), the antimetabolite 3-acetylpyridine (Sapolsky, 1985a, b), hypoxia – ischemia (Sapolsky and Pulsinelli, 1985; Koide et al., 1986; James Davis, personal communication) and the oxygen radical generator paraquat (Sapolsky et al., 1988) are worsened by GCs. In vivo, reduction of GCs (by adrenalectomy) reduces hippocampal damage, while elevating GC concentrations to the stress range (via GC administration) increases damage. The extremes vary as much as 10-fold, emphasizing the potency of this effect.

These studies on the GC exacerbation of the toxicity of varied insults make it uncertain what the precise role is of the steroids in hippocampal degeneration during aging. Do the GCs *kill* hippocampal neurons outright, or do they *endanger* them and compromise their ability to survive insults? The latter would appear to be unlikely, given that GCs contribute to hippocampal senescence even in the absence of overt neurological crises such as hypoxia – ischemia or seizure. However, the GCs may induce a sufficient vulnerability in the neurons such that the insults that now become damaging are so subtle as to be difficult to detect – perhaps a brief period of vasospasm, or the mild hypoglycemia induced when an animal technician feeds the rats late may be sufficient to damage an aged hippocampal neuron, with its lifetime of cumulative GC exposure. As is obvious, the latter model – one of an extremely lowered threshold for damage – would be extremely difficult to test, especially in the prolonged circumstances of an aging study. The question is not yet settled.

Features of glucocorticoid endangerment of hippocampal neurons

In attempting to uncover the mechanisms by which GCs endanger the hippocampal neurons, it became important to determine whether it was the GC molecule itself which acted upon the neuron to endanger it, or whether the GCs' effects arose secondarily, from one of their many peripheral actions. One study implicating GCs in damaging the hippocampus suggested that it was via secondary GC actions. Specifically, the authors suggested that it was not the chronic hippocampal exposure to GCs themselves which was destructive, but rather the chronic diminution of ACTH secretion (which is typically inhibited by elevated GC concentrations, via the negative feedback effects of the steroid (Keller-Wood and Dallman, 1984). Conversely, given this view, the protective effects of long-term

adrenalectomy were not so much from the elimination of the GCs, but rather from the resultant elevation of ACTH concentrations. In support of that idea, it was shown that sustained exposure of aging rats to an ACTH analogue protected the hippocampus in much the same manner as did adrenalectomy (Landfield et al., 1981). In contrast to the predictions of that scenario, however, is the fact that in the aging rat, ACTH concentrations are elevated and, in fact, are the driving force for the elevated basal GC concentrations (Tang and Phillips, 1978). As an alternative model that we have favored, GCs could be endangering the neurons directly, independent of any secondary physiological effects. The first support for this view was somewhat indirect – the GC exacerbation of damage by the various insults was exclusive to, or occurred preferentially in the hippocampus. The atypically high concentration of corticosteroid receptors in that structure suggested a direct, receptor-mediated GC effect. Stronger support for the view of direct GC endangerment comes with the observation that GCs can exacerbate the damaging effects of these various insults when studied in vitro with primary hippocampal cultures (Sapolsky et al., 1988). Much as with the in vivo model, damage was exacerbated by concentrations of GCs that were, themselves, not toxic. Hypothalamic or cerebellar cultures were not sensitive to the GC insult synergy. The endangerment was not triggered by other steroids such as testosterone, estradiol or progesterone. This suggests that it is not a generalized steroid effect, but rather is specific to GCs, and mediated by their receptors. This was further supported by the finding that the GC endangerment could be prevented with GC receptor antagonists in the culture system (in preparation).

In then exploring the mechanisms by which the GCs might endanger the hippocampus, it became important to test whether a very attractive model of cell death was relevant. One of the hallmarks of the stress-response is the suppression of immune function, and GCs are central to this process. Among the numerous catabolic actions of GCs in the immune system, the steroids can trigger lysis of lymphocytes and thymocytes (reviewed in Munck et al., 1984; Bateman et al., 1989). Recent work has uncovered the mechanism by which GCs trigger this instance of "apoptosis", or programmed cell death. The steroids induce the synthesis of protein(s); it is either an endonuclease itself, or activates an endonuclease. The result is that DNA is cleaved at internucleosomal sites, producing a characteristic "ladder" of DNA fragments which are multiples of 180 base pairs (Wyllie, 1980; Umansky et al., 1981; Cohen and Duke, 1984; Compton and Cidlowski, 1986, 1987; Compton et al., 1988). The DNA fragmentation precedes the loss of cell viability. Apparently, during this process, the cell even attempts to repair its DNA in a futile attempt to stop the degradation; as evidence, poly(ADP-ribosyl)ation is activated, NAD concentrations fall, and the GC-induced lymphocytolysis is exacerbated by inhibition of poly(ADP-ribose)synthetase (Wielckens and Delfs, 1986). Moreover, it appears that this mechanism can potentially be provoked in a broad range of cell types, since cytotoxic T cells also promote DNA cleavage in target cells (Ucker, 1987). These observations suggested that GCs might provoke a similar process in the hippocampus. However, we have recently obtained evidence suggesting that this is not the case. Under conditions in which GCs exacerbate the damaging effects of the excitotoxin kainic acid, we observed no evidence of DNA cleavage. Moreover, the viability of the hippocampal neurons was not exacerbated by inhibition of poly(ADP-ribose)synthetase (Masters et al., 1989). In hindsight, this finding seems reasonable and comforting. Instances of apoptosis – during development, immunosuppression, cytotoxic attack – tend to be rapid, all-or-none in nature, and highly stereotyped in the patterns of damage. It seems logical that the underlying mechanisms should be fairly discrete, linear cascades of damage. In contrast, the GC toxicity during aging, and the insults whose toxicities are exacerbated by GCs, emerge slowly, are more selective, and are relatively ideosyncratic. Teleologically, it would

seem surprising if the underlying mechanism would turn out to be a fairly discrete "suicide switch". The present evidence suggests that the mechanisms of GC endangerment are anything but discrete.

Energy availability and the glutamatergic synapse

How then do GCs compromise neuronal viability? The insults made worse by GCs are extremely varied in their mechanisms of action – cutting off of oxygen and glucose availability to the brain (hypoxia – ischemia), greatly accelerating the rate of action potentials (seizures), disrupting energy production by uncoupling electron transport (3-acetylpyridine). This suggests that whatever the GCs are doing, it is a broad and generalized vulnerability that they are inducing. Yet, as will be reviewed here, these differing neurological insults appear to have two common threads. All appear to damage hippocampal neurons via a cascade that involves excessive synaptic concentrations of excitatory amino acid neurotransmitters which, through the NMDA receptor, leads to mobilization of damaging concentrations of free cytosolic calcium. Second, this excitatory/calcium cascade seems to be highly sensitive to energy depletion. Very recent work suggests that the GC endangerment is involved with both of these themes.

Currently, there is tremendous excitement concerning the role of glutamate and other excitatory amino acid neurotransmitters (EAAs) as a common pathway by which hypoxia – ischemia, seizure and hypoglycemia are neurotoxic. The hippocampus is rich in EAAs and their receptors, which include the kainic acid, quisqualate, and *N*-methyl-D-aspartate (NMDA) receptor. The NMDA receptor is immensely complex; there is an allosteric binding site for glycine, and a receptor-gated channel that allows the flow of sodium and calcium only under conditions of depolarization, since at resting potential, the channel is typically blocked by magnesium in a voltage-dependent manner (MacDermott and Dale, 1987). It is the NMDA receptor which has been most implicated in neuronal damage. The EAAs can be neurotoxic, and their synaptic concentrations rise to excessive levels following hypoxia – ischemia, hypoglycemia or seizures. These excessive levels appear both as enhanced release of the EAAs from the presynaptic neuron, as well as failure of reuptake back into the neuron, or of uptake into glia. Commensurate with the excitatory effects of these EAAs, all of these insults lead to a period of neuronal excitation. As the most direct evidence available that the EAAs mediate the neurotoxicity of these insults via the NMDA receptor, damage induced by hypoxia – ischemia, incomplete ischemia, anoxia (in cultured neurons), excitotoxic seizures, brain trauma, hypoglycemia and electron transport uncouplers can all be prevented by silencing the NMDA synapse. This can be done with antagonists that directly block the NMDA receptor, such as aminophosphonovaleric acid (APV). This can also be brought about with non-competitive antagonists that block the receptor-gated channel (such as magnesium, MK-801, dextromethorphan, or dissociative anesthetics such as phencyclidine or ketamine). Finally, it can be brought about by destruction of glutamatergic projections to the vulnerable neurons. With these varied paradigms, there is usually protection, as measured by the numbers of volume of neurons lost, by biochemical or electrophysiological markers of injury, or by functional indices of damage. Collectively, these numerous studies represent a near consensus that EAAs, via the NMDA receptor, can mediate much of the damaging effects in the hippocampus of these neurological insults (reviewed in Rothman and Olney, 1987; Choi, 1988).

The most accepted mechanism by which EAA-activation of the NMDA receptor damages neurons is via increased free cytosolic concentrations of calcium. Both EAAs and the varied insults discussed increase calcium conductance, decrease extracellular calcium concentrations, and cause intracellular calcium accumulation, and these effects can be blocked with NMDA receptor antagonists. Moreover, the toxicity of EAAs and these various

insults in vitro is diminished by removing calcium from the medium (cf. Choi, 1988). There are a number of possible routes by which the calcium is mobilized. Of primary interest is the calcium channel gated to the NMDA receptor. In addition, the depolarization by the EAAs will lead to opening of voltage-gated calcium channels. As an additional mechanism, the excessive sodium influx during sustained depolarization will increase free cytosolic calcium via a cell surface membrane sodium/calcium exchanger, as well as via similar exchangers that liberate calcium from organelles. Finally, the sequestering and efflux of the calcium is apparently impaired during these insults. Thus, the excessive cytosolic calcium concentrations are derived from both increased entry of the ion into that cellular compartment, as well as impaired removal from it (McBurney and Neering, 1987; Choi, 1988). Excessive levels of such calcium are among the broadest of routes by which cells can be damaged, potentially via generation of oxygen radicals, and activation of proteases, lipases and nucleases (Cheung et al., 1986). At present, it is not clear which routes of damage are relevant in these cases of neurological crisis.

Thus, considerable evidence suggests that hypoxia – ischemia, hypoglycemia and seizure damage the hippocampus via this EAA/NMDA/calcium cascade. Critically, the cascade seems extremely sensitive to energy depletion. All three of the insults deplete hippocampal neurons of energy, as measured by declining ATP and phosphocreatine concentrations. In general, the order of severity of depletion is hypoxia – ischemia > hypoglycemia > seizure (Auer and Siesjo, 1988). Moreover, the damaging effects of all of the insults can be attenuated by supplementing neurons with energy. That is obviously the case with hypoglycemia, where the insult is, by definition, attenuated with additional glycose. It is also the case with seizure where limited energy availability compromises neuronal viability (Meldrum, 1983; Johansen and Diemer, 1986; Sapolsky and Stein, 1989). Hypoxic – ischemic damage also seems sensitive to energy, although the issue there is complicated by the issue of lactate-induced acidosis. A long-standing observation is that hypoxic – ischemic damage to the hippocampus is exacerbated in vivo by glucose pre-loading (Myers and Yamaguchi, 1977; Siemkowicz and Hansen, 1978; Ginsberg et al., 1980; Kalimo et al., 1981; Pulsinelli et al., 1982). The most convincing explanation for this is that this allows the neuron to enter the crisis with more glycogen stores and thus carry on more anerobic metabolism during that time. A major consequence of this is elevated lactate production, leading to damaging acidosis. As evidence for this scenario, exposure of normoxic neurons to the concentrations of lactate or hydrogen ions generated during such hypoxia – ischemia is damaging (Kraig et al., 1987). In this view then, hypoxia – ischemia is damaging not so much for its disruption of energy production, but for the kind of energy produced during the crisis. This is turning out to be somewhat of a simplification, however, and there is also a route of damage during hypoxia – ischemia which is energy-dependent; this has most readily been demonstrated with in vitro systems of primary dispersed neurons or hippocampal slices, and where the insult is anoxia, rather than hypoxia. With these experimental paradigms, under circumstances where the acidosis is, itself, not sufficient to be damaging, glucose can protect against both neuron death and dysfunction (Schurr et al., 1987; Tombaugh and Sapolsky, 1988).

Thus, all of these insults seem to damage, in at least in part, because of their disruptive effects upon neuronal energetics. It is clear that such disruptions exacerbate the damaging effects of the glutamate/NMDA/calcium cascade just outlined. There are numerous mechanisms potentially underlying this. To begin with, glutamate release will be enhanced and uptake blocked. The latter can fail in at least two ways. Pre-synaptic reuptake is mediated by a glutamate/sodium cotransporter, which is driven by a steep extracellular/intracellular sodium gradient. With energy failure, Na-K-ATPase fails, the gradient is lost, and reuptake fails; for example, this occurs after

hypoxia – ischemia (Drejer et al., 1985). As evidence of the importance of this component, EAA toxicity is enhanced when neuronal reuptake is blocked (either by poisoning the pump, or destruction of pre-synaptic neurons (Kohler and Schwarcz, 1981; Choi, 1987b)). In addition, glial uptake is also dependent on energy (as manifested by maintaining gradients of both sodium and potassium in order to transport glutamate (Barbour et al., 1988)). Thus, various NMDA-mediated insults to neuronal cultures are more toxic in the absence of glia (Vibulsreth et al., 1987). *Enhanced release* occurs in at least two ways. With energy scarcity neurons depolarize, increasing calcium-dependent release of EAAs. Second, the sodium/glutamate cotransporter used for neuronal reuptake is bidirectional; with failing energy and increased intracellular sodium concentrations, the transport reverses, causing calcium-independent EAA leakage (Drejer et al., 1985; Dagani and Erecsinka, 1987). Evidence suggests that the calcium-independent component is the major route by which energy failure leads to excessive release of glutamate. Thus, a paucity of energy will enhance glutamatergic tone in a variety of ways. The implications of this are shown most explicitly with the recent demonstration in cerebellar cultures that glutamate toxicity via the NMDA receptor is energy-dependent (Novelli et al., 1988).

Energy failure is likely to lead to more post-synaptic calcium mobilization, simply because of more EAA interaction with the NMDA receptor. There are many reasons to believe that the calcium signal will be further enhanced because of energetic constraints in the post-synaptic neuron itself: (1) The calcium channel gated to the NMDA receptor is blocked by magnesium in a voltage-dependent manner. With depolarization in states of energy depletion, the blockade is released, allowing enhanced calcium influx. (2) With loss of the membrane potential, intracellular sodium concentrations rise, releasing calcium from intracellular storage sites, via a sodium/calcium exchanger. (3) Sequestering of calcium into mitochondria or SER will be compromised, as the process is energy-dependent (relying either on ATP directly, or on a steep sodium gradient). (4) The efficiency of efflux should also be compromised, as it is similarly energy-dependent. Moreover, one route of efflux is through a sodium/calcium exchanger; as intracellular sodium concentrations rise, not only should calcium efflux be blocked, but the exchanger can reverse, providing an additional route of calcium influx (reviewed in McBurney and Neering, 1987; Blaustein, 1988; Choi, 1988).

Glucocorticoids and the glutamatergic synapse

This preceding section has outlined, at some length, the considerable evidence that hypoxia – ischemia, seizure and hypoglycemia damage the hippocampus via the interacting components of energy depletion and the glutamate/NMDA/calcium cascade. We are beginning to obtain evidence that GCs exacerbate these three insults by exacerbating this cascade.

The first potential route by which this might occur is rather specific to glutamate trafficking in the synapse. A large percentage of neurotransmitter glutamate cycles through a shuttle involving astrocytes. Synaptic glutamate, after dissociating from the post-synaptic receptor, can be taken up by glia and converted to glutamine (by the enzyme glutamine synthetase), which is then shuttled back to the pre-synaptic neuron for conversion to glutamate. This shuttle has been viewed as a protective mechanism to remove highly excitatory glutamate from the synapse and return it to the neuron as "deactivated" glutamine (Hertz et al., 1983). The potential for the glutamine to be converted back to glutamate is shown with the recent demonstration that glutamine increases anoxic damage to cortical cultures, and this augmentation is NMDA receptor-dependent (Monyer et al., 1988). It has been estimated that as much as 80% of neurotransmitter glutamate cycles this way (Hertz et al., 1983). This suggests that the total availability of glutamine to neurons via this cycle is an important determinant of the amount of

glutamate that the neurons can release. This is supported by several recent studies. In one, glutamate release from striatal slices increased when the glucose concentrations in the media were dropped. This fits nicely with the previous discussion about EAA release being exacerbated by energy failure. Importantly, this hypersecretion could only be sustained when the neurons were supplied with glutamine (Szerb, 1988). In another study, when glutamine cycling was blocked with the glutamine synthetase inhibitor methionine sulfoximine, glutamate release declined (Rothstein and Tabakoff, 1985).

Thus, given that EAA release will be exacerbated by energy failure and neurological insults, the extent of hypersecretion can be sensitive to the extent that glutamine can be generated and supplied to the neurons. In that context, it is of importance to note that GCs stimulate the synthesis of glutamine synthetase, the rate-limiting state in glutamine production. This is a well-characterized genomic effect in the developing brain, and in both developing and adult muscle (Martinez-Hernandez et al., 1977; Pishak and Phillips, 1980; Holbrook et al., 1981; Juurlink et al., 1981; Kumar et al., 1984; Smith et al., 1984; Patel and Hunt, 1985; Max et al., 1987); less attention has been focused on whether this also occurs in the adult brain. However, it has recently been shown that GCs increase amounts of mRNA for activity of glutamine synthetase in the adult hippocampus (Nichols et al., 1989; Tombaugh and Sapolsky, in preparation). This suggested that GCs could potentially endanger the hippocampus by indirectly fueling the generation of more glutamate. However, we are not sanguine about the importance of this regulatory link in the adult hippocampus. In the two studies just cited, animals were exposed to GC concentrations in the upper (stressed) physiological range constantly for days. Under such conditions, hippocampal glutamine synthetase activity was increased by approximately 25%, a not particularly robust amount. We then examined whether more physiological circumstances of GC hypersecretion could increase the enzyme's activity. We observed that one day of status epilepticus seizures (which provokes approximately 12 h of GC hypersecretion; Stein and Sapolsky, 1988), and three days of intermittent and shifting stressors both failed to significantly elevate glutamine synthetase activity (while being sufficient to cause moderate rises in muscle activity of the enzyme). Thus, our present sense is that while this could theoretically be a route for GC's exacerbating the glutamate/NMDA/calcium cascade, it can only apply to stressors more severe or prolonged than these rather major ones.

The most plausible route by which GCs endanger the hippocampus is by disrupting energy storage. Previous work implied this (Sapolsky, 1986b). In it, I reasoned that the common thread among the insults worsened by GCs was a problem of energy – either pathologic disruption of energy production (hypoxia – ischemia, antimetabolites), or pathologically-elevated demands for energy (excitotoxic seizures). I reasoned that the broad vulnerability induced by GCs might be an energetic one; supporting this, I found that supplementing neurons with energy substrates such as glucose, mannose or ketone bodies reduce the endangering effects of GCs (Sapolsky, 1986b). We subsequently found that this also worked in vitro (Sapolsky et al., 1988). What remained was to demonstrate how GCs disrupt hippocampal energetics. The hormones appear to inhibit glucose transport; this is not a particularly radical observation, given that GCs have long been known to inhibit glucose transport in peripheral tissues (Munck, 1971), and the molecular biology of the effect is well-characterized (Horner et al., 1987). The first evidence that this was occurring in the brain was the finding that GCs inhibit local cerebral glucose utilization in many brain regions, with the hippocampus being among the most sensitive (Bryan and King, 1988; Kadekaro et al., 1988). A number of mechanisms could account for this, and it could be occurring at a number of possible cell types. Clarifying these issues, we have found that nanomolar concentrations of corticosterone (the rat GC) inhibit approximately 20 – 35% of glucose

transport in both cultured hippocampal neurons and glia (Horner and Sapolsky, 1988). This inhibition is time- and protein synthesis-dependent. It is not triggered by non-GC steroids, but is triggered by other GCs, such as cortisol and dexamethasone. The inhibition is mediated by the type II GC receptor; as evidence, the type II ligand dexamethasone inhibits glucose transport, 50% inhibition occurs around the Kd of the receptor, and the inhibition is blocked with a type II receptor antagonist. Finally, the effect does not occur in cultures from other brain regions. The steroid and receptor specificity, and protein synthesis dependence agree with what is known about GC inhibition of glucose transport in peripheral tissues (Munck, 1971).

Neurons are notorious for their metabolic vulnerability – they consume enormous amounts of energy, it must come almost exclusively in the form of glucose, and it is not stored as glycogen in particularly large amounts (Siesjö, 1981). Nevertheless, it seems unlikely that a 20 – 35% inhibition of glucose transport is sufficient to, in effect, get the hippocampus into trouble under resting conditions. However, a similar inhibition during a period of major energetic crisis for the neuron – i.e., one of the neurological insults discussed – could well exacerbate the insult. Critically, the consequences of the small inhibition of glucose transport need not be dramatic; it must merely be multifaceted. At the pre-synaptic end of the picture, a bit more EAAs may be released and a bit less taken up again. Neighboring glia may be somewhat impaired in their ability to take up EAAs, to act as a proton sink, or to detoxify ammonia. Finally, at the post-synaptic end, there might be a small bias towards more calcium entering the free cytosolic compartment, less being cleared from it and less export of protons. Collectively, these steps may bias a neuron in energetic trouble towards death, rather than survival. If so, this would be a metabolic explanation for the capacity of GCs to synergize with these insults under conditions where the GCs are, themselves, not damaging. Only a few of these steps have been tested yet, but they support this scenario. In one study, GCs appeared to inhibit glutamate reuptake in the hippocampus (Halpain and McEwen, 1988). In addition, GCs increase calcium-dependent afterhyperpolarizations in hippocampal pyramidal neurons, accounting for the phenomenon in the aged hippocampus (Kerr and Landfield, 1988). Neither of these studies examined whether the GC effects arise from their effects on glucose transport or from any of their many other actions. Clearly, a great deal of work needs to be done to test the ideas just outlined.

The most specific prediction from these ideas is that if GCs endanger hippocampal neurons by exacerbating the glutamate/EAA/calcium cascade, GC endangerment of the hippocampus should be reversed by blocking the NMDA receptor. We have recently observed just that (Armanini et al., 1989). In order to test this, we had to find an insult which was exacerbated by GCs, but was not, itself, working via the NMDA receptor; we would then see if receptor blockade would subtract out the GC component of the synergy. We tested 3AP, the electron transport uncoupler. At a high concentration, the hippocampal damage that it induced was indeed NMDA receptor-dependent and was reversed by APV. We interpreted this as showing that the profound energy failure caused by the high 3AP concentrations triggered the glutamate/EAA/calcium cascade. At lower concentrations of 3AP, however, lesser but still consistent hippocampal damage occurred, which was insensitive to APV. This implies an additional mechanism by which 3AP damages, which is the predominant route at these lower concentrations. Working with this lesser 3AP insult, we replicated our prior observation that GCs synergized with the toxin. Specifically, the volume of hippocampal damage in animals exposed to high physiological GC concentrations for a week before and after the insult was about 75% greater than in rats adrenalectomized and GC-free during that period. We then found that microinfusion of APV completely eliminated the GC exacerbation of damage; again, this was without changing the toxicity of 3AP in the adrenalectomized rats. Although preliminary, this represents strong support for

the model of GCs exacerbating the glutamate/NMDA/calcium cascade. We are currently pursuing the mechanisms underlying this exacerbation, along the lines outlined above.

Conclusions

These studies suggest that GCs, at least in part through their disruptive effects on glucose transport, leave hippocampal neurons in a state of metabolic vulnerability. In the absence of a co-incident metabolic challenge, this vulnerability is survived readily. However, when co-incident insults occur, neuronal viability is compromised, at least in part via exacerbation of the EAA cascade of damage. These observations are of some potential relevance, in that they suggest that exogenous GCs, in the aftermath of some insults, can potentially exacerbate hippocampal damage, and should be avoided if possible. Moreover, they suggest that strategies to decrease endogenous GC secretion after such insults, or over the course of the lifespan, can protect the hippocampus from damage. It has recently been shown that inhibiting the GC stress response in the aftermath of status epilepticus seizures, by administering the adrenal steroidogenesis inhibitor metyrapone, diminishes hippocampal damage (Stein and Sapolsky, 1988). In an approach meant to decrease the total lifetime exposure to GCs, we demonstrated that a neonatal behavioral intervention which reduces adult basal GC concentrations in the rat prevents some the neuron loss and spatial learning deficits that characterize aging in the rat (Meaney et al., 1988). In considering whether these interventions may be of any clinical relevance, the question remains whether any of the findings described here apply to the human hippocampus. This remains an open question at this point, and should be a focus of research in coming years.

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