From neurotoxin to neurotrophin

Christian Mirescu & Elizabeth Gould

Glucocorticoids are important for neuronal function, but their release in conjunction with a brain injury can intensify neuronal death, causing more harm than good. A new study shows that delivery of genes that have been modified to alter glucocorticoid signaling can block the toxic effects of the hormone *in vitro* and *in vivo*, converting what was once a neuron's worst enemy into its best friend.

Nature is conservative: the same signaling molecules exist across phyla and in different systems, where they may even have opposing roles. For example, adrenal steroids have many protective and beneficial actions in the brain—removal of the adrenal glands results in apoptosis of dentate gyrus granule cells[1] and decrements in synaptic plasticity and learning[2,3]. But under conditions of stroke, seizures or brain injury, glucocorticoids are toxic for cell survival, in particular within the hippocampus[4–6]. Thus, in treating these conditions, the difficulty lies in eliminating the detrimental effects of glucocorticoids while preserving their favorable actions. In this issue, Kaufer *et al.*[7] show that targeting glucocorticoid signaling through gene delivery is an effective and potentially promising approach to deal with this problem.

The authors devised three viral vector strategies to counteract the deleterious effects of corticosterone (the main rodent glucocorticoid). First, they designed a vector to overexpress 11β-hydroxysteroid dehydrogenase type 2, a renal enzyme responsible for metabolizing corticosterone into inactive cortisone, thereby diminishing glucocorticoid action. Second, the authors created a vector with a dominant-negative form of the glucocorticoid receptor (GR). This would attenuate glucocorticoid receptor signaling without affecting the beneficial actions of mineralocorticoid receptors (another type of brain receptor responsive to corticosterone). Finally, the researchers coupled the glucocorticoid receptor ligand-binding domains with the DNA-binding domains of the human estrogen receptor (ER). Because estrogen has neuroprotective effects[8], this vector containing an ER/GR 'chimera' receptor not only should block the toxic effects of glucocorticoids (through competition with native glucocorticoid receptors) but should also elicit estrogenic neuroprotection in the presence of corticosterone.

The authors tested the efficacy of these vectors in modulating the toxic actions of glucocorticoids by delivering the modified genes into cultured hippocampal neurons. In the presence of glucocorticoids, cultured hippocampal cells are more sensitive to the damaging effects of excitotoxins such as kainic acid[4,9]. Kaufer *et al.*[7] found that each of their vectors significantly enhanced cell survival in cultures treated with kainic acid and corticosterone. Although the vectors blocked the contribution of glucocorticoids to kainic acid toxicity, they did not prevent the negative effects of kainic acid itself, confirming that the vectors were specifically altering glucocorticoid signaling and not having a general neuroprotective effect.

Normally, glucocorticoids downregulate expression of brain-derived neurotrophic factor (BDNF), whereas estrogens upregulate it[7]. When the authors infected hippocampal cells with the ER/GR vector, the cells responded to glucocorticoid treatment

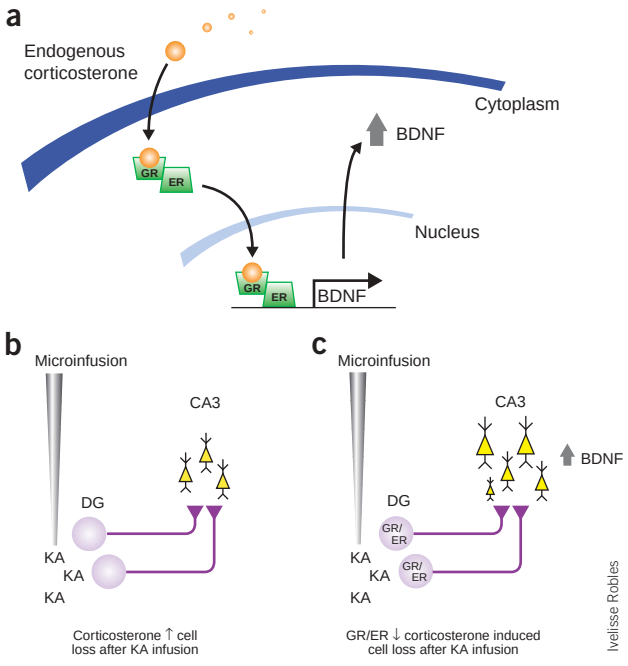

Figure 1 Gene delivery protects neurons from glucocorticoid enhancement of excitotoxic cell death in the hippocampus. (**a**) Expression of the ER/GR chimeric receptor in cultured hippocampal neurons alters the glucocorticoid corticosterone signaling pathway and increases BDNF expression. (**b**) Corticosterone enhances the cell death of CA3 neurons induced by infusion of the excitotoxin kainic acid (KA) into the dentate gyrus of rats. (**c**) Delivery of the GR/ER vector along with KA diminishes the neuronal damage in CA3 caused by corticosterone, possibly through neuroprotection by increased BDNF.

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with the genetically engineered estrogenic response—they produced more, not less, BDNF. This provided an elegant confirmation that the transgenes were altering hormonal responses in the intended direction. Furthermore, BDNF alone could partially block the effects of the toxic cocktail of kainic acid and glucocorticoids, suggesting that the ER/GR vector protected hippocampal cells, at least in part, by enhancing BDNF expression.

To test whether their gene-delivery approach could modulate glucocorticoid responses *in vivo* during and immediately after brain damage, the authors infused kainic acid directly into the hippocampus simultaneously with one of the three vectors. In all cases, they found that vector administration significantly attenuated the neurotoxic actions of kainic acid, decreasing the size of the lesion and the loss of neurons in the hippocampus, similar to the effects observed *in vitro*. The ER/GR vector had the largest beneficial effects, probably because of its actions on the neurotrophin BDNF. The protection offered by minimizing or redirecting glucocorticoid signaling is remarkable, as the vectors were delivered not days before injury but rather during injury.

For unknown reasons, the virus used to construct these vectors (herpes simplex virus 1) preferentially infects dentate gyrus granule cells, which are relatively resistant to glucocorticoid-induced damage. Indeed, the granule cell population, probably because of its ability to undergo neurogenesis in adulthood, is often less damaged than the pyramidal cell population by stroke, epilepsy or Alzheimer disease[10–12]. At first glance, then, the granule cells seem to be the least needy neuronal population to target for neuroprotection. However, granule cells modulate the survival of their relatively vulnerable targets, CA3 pyramidal cells, and so this selective infection is actually fortuitous. Kaufer *et al.*[7] suggest a scenario in which ER/GR-infected granule cells respond to glucocorticoids with an increase in BDNF expression; this, in turn, protects their target cells from death (**Fig. 1**).

As with any pioneering effort, a number of problems must be solved before this approach could be useful clinically. To be effective, these vectors must be infused directly into the brain region of interest, requiring brain surgery, which has negative consequences independent of the specific experimental manipulation. Clearly, therapeutic usefulness of this approach would require the development of noninvasive methods for vector delivery. Another limitation is the current inability to infect specific types of neurons in designated areas at will. As explained above, the viral vector used in the present study preferentially infects dentate gyrus granule cells. Thus, the extent to which a similar approach could be used for protecting neuronal populations in other brain regions is unclear.

Kaufer *et al.*[7] have demonstrated the feasibility of genetically manipulating the response of a complex signaling molecule, corticosterone, to inhibit its neurotoxic effects in the presence of an exogenous excitotoxin. These intriguing findings prompt additional questions. For instance, can such an approach be used to prevent or diminish other adverse effects of glucocortoids, such as those that arise following stress? Would infusion of these vectors prevent the negative consequences of chronic stress in adulthood, such as atrophy and death of CA3 pyramidal cells[2,3], or eliminate the effects of early life stress, such as glucocorticoid hypersensitivity, increased anxiety, impaired learning and diminished adult neurogenesis[13,14]? If so, then it is tempting to speculate that a similar approach might be useful, in principle, for the treatment of stress-related psychiatric conditions such as depression or anxiety disorders. Clearly, much work remains, but Kaufer *et al.*[7] have nevertheless opened the door for the potential use of gene therapy in managing the adverse consequences of glucocorticoids.

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Crossing the boundaries of sensory neurogenesis

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Boundary cap cells of the dorsal root ganglia were thought to be limited to a structural role regulating migration into or out of the neural tube. Now a study in this issue reports that they are progenitors of small-diameter nociceptive neurons.

Different subsets of neural crest cells are specified to take different migration paths and to acquire different fates. Neural crest cells immigrate into the sensory dorsal root ganglia (DRG) in two successive waves, giving rise to distinct subsets of sensory neurons[1]. These waves have been distinguished based on differences in their expression of neurogenic genes, but the origin of the cells that form each subset of sensory neurons has been uncertain. In this issue, Maro and colleagues show that the boundary cap cells, which are derived from neural crest and define the points at which axons pass into or out of the spinal cord, themselves give rise to a late-migrating wave of progenitors that forms neurons and glia within sensory ganglia[2]. This unexpected observation provides an important insight into the identity of the progenitors that generate a subset of sensory neurons, while raising new questions.

The first wave of neurogenesis occurs in the DRG between E9.5 and E11.5, forming the large-diameter sensory neurons, including mechanoreceptive (stretch-sensing) neurons that express the BDNF receptor TrkB and proprioceptive (limb position–sensing) neurons that express the NT-3 receptor TrkC (**Fig. 1**). The second wave of neurogenesis occurs between E10.5 and E13.5, forming the small-diameter nociceptive (pain-sensing) neurons that express the NGF receptor TrkA, as well as

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