acids and suggested that Asp57 and Asp149 are less than 5 Å away from Lys279. Second, by replacing the charged amino acids with cysteine residues, Kash *et al.* found that it was possible to form disulphide bonds between them, pointing again to the close proximity of these amino acids. Intriguingly, the formation of disulphide bonds between Asp149 and Lys279 depended on the presence of the agonist GABA. By contrast, Asp57 and Lys279 could be linked independently of agonist binding. It is therefore possible that Asp149 and Lys279 move closer to each other after agonist binding, and that this movement is the crucial link between binding and channel opening. It will now be necessary to explore what happens downstream of this interaction — at the site of the channel gate itself.

Juan Carlos López

References and links

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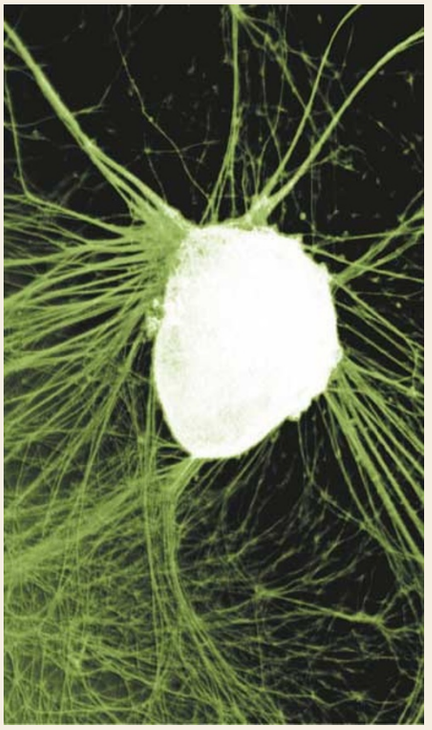


A mouse dorsal root ganglion explant showing neurite outgrowth that was stimulated by engagement of the Fas receptor. Reproduced , with permission, from Desbarats *et al.*, *Nature Cell Biology* © (2003) Macmillan Magazines Ltd.

NEUROLOGICAL DISORDERS

Caspase, the friendly protein

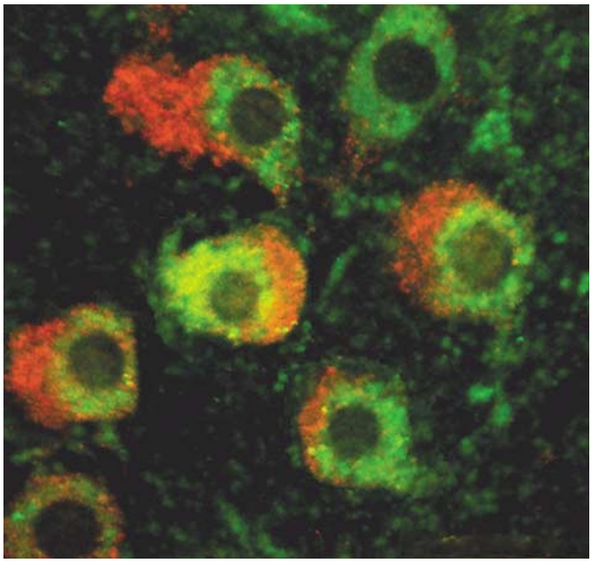
Activation of caspase 3 has become almost synonymous with cell death. So it comes as a surprise to discover that caspase 3 cleavage seems to be an essential part of a neuroprotective effect known as preconditioning. Experiments described by McLaughlin *et al.* in *Proceedings of the National Academy of Sciences of the USA* point towards a potential mechanism for preconditioning that relies on limited activation of caspase 3 to induce subsequent neuroprotection.

Exposure to a limited insult — such as a transient ischaemia — can protect neurons against later, more severe challenges. This preconditioning effect requires protein synthesis and is known to depend on processes that are normally associated with the early stages of apoptotic pathways. For example, metabolic dysfunction leading to the opening of ATP-sensitive K^+ channels (K_{ATP}) and production of reactive oxygen species (ROS) can contribute to preconditioning, as can the activation of heat-shock proteins (HSPs).

The new study shows that, during preconditioning, the 'cell-death' pathways proceed considerably further. In both *in vivo* and *in vitro* models of preconditioning, the authors found that a preconditioning treatment led to cleavage of caspase 3, and that the cleaved caspase 3 was present in neurons during the time period of maximum neuroprotection. But despite the presence of cleaved caspase 3, the preconditioned neurons showed no signs of apoptotic cell death.

To investigate the role of caspase 3 in neuroprotection, McLaughlin *et al.* tried blocking various stages of the apoptotic pathway in their *in vitro* model. Blocking K_{ATP} activation or caspase cleavage, or applying ROS scavengers, prevented the neuroprotective effect. ROS scavengers or K_{ATP} blockers also prevented the cleavage of caspase 3, indicating that caspase 3 activation depends on both ROS and K_{ATP} activation.

What happens next? Although Bcl-x_L (an anti-apoptotic protein) was induced during preconditioning, its induction did not share key properties with other aspects of preconditioning, making it unlikely that it is responsible for neuroprotection. A better candidate is HSP70, a chaperone protein that is also induced. Treatments that block preconditioning in the *in vitro* model — including prevention of caspase 3 cleavage — also blocked induction of HSP70. The authors propose a model for preconditioning in which the initial insult activates ROS and K_{ATP}, leading to cleavage of caspase 3. The cleaved caspase 3 binds to the HSP70 homologue HSC70 (heat-shock cognate 70kDa), which is constitutively expressed, and this binding prevents caspase activation from leading to apoptosis. The expression of HSP70 would be induced by a feedback loop resulting from the depletion of HSC70 and other caspase-binding proteins, and the increase in HSP70 would subsequently protect cells against damage.



Colocalization of caspase 3 and HSP70. Image courtesy of B. McLaughlin, Vanderbilt University, Nashville, Tennessee, USA..

In support of this hypothesis, the authors found that exposing cultured cells to excess HSC70 prevents the increase in HSP70 that results from preconditioning, and that this treatment also blocked the neuroprotective effect. Clearly, more work is needed to test the hypothesis and in particular to show that the proposed mechanisms are active *in vivo* and in other forms of preconditioning. But greater understanding of the ways in which neurons can protect themselves against damage has the potential to lead to new targets for neuroprotective therapies. As the authors point out, the idea that apoptotic pathways might lead to the induction of neuroprotective mechanisms might also necessitate a re-evaluation of therapies that are designed to block these pathways.

Rachel Jones

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