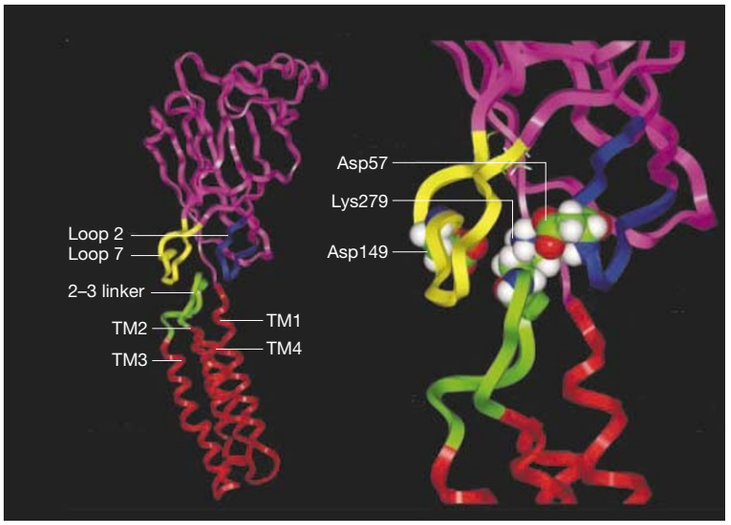
ION CHANNELS

Charging at the gate



Molecular model of the $GABA_A$ receptor, showing the positions of the loops that seem to interact during gating. The close-up on the right shows the location of the key interacting residues. TM, transmembrane domain. Reproduced, with permission, from Kash *et al.*, *Nature* © (2003) Macmillan Magazines Ltd.

How agonist binding leads to opening of the pore remains one of the outstanding questions about ligand-gated ion channels. Reporting in *Nature*, Kash *et al.* now provide compelling evidence that specific electrostatic interactions between residues of the extracellular domain and the linker between two transmembrane domains are crucial for gating of the $GABA_A$ (γ-aminobutyric acid) receptor channel.

Their starting point was an acetylcholine-binding protein from the snail *Lymnaea stagnalis*. This protein is structurally homologous to the extracellular domain of many ligand-gated ion channels (including the $GABA_A$ receptor), and its crystal structure is available. So, the authors aligned the two molecules and identified two flexible loops — loops 2 and 7 — that were ideally positioned to interact with the segment of the channel that links its second and third transmembrane domains — the 2–3 linker. Loops 2 and 7 have several negatively charged amino acids, whereas the 2–3 linker has two basic residues. Kash *et al.* therefore wondered whether electrostatic interactions between some of these amino acids participated in gating. They mutated the different acidic residues of loops 2 and 7, and found that replacing Asp57 or Asp149 with lysine residues significantly affected gating. But more importantly, they found that if Lys279 in the 2–3 linker was simultaneously replaced with an asparagine residue, then the channel behaved normally, pointing to the existence of direct interactions between the positively and negatively charged amino acids.

Two additional experiments provided further evidence for this interaction. First, Kash *et al.* used a technique known as double-mutant cycle analysis. Double-mutant cycles are normally used to determine the interaction between two residues in a folded protein, relative to their interaction in the unfolded state. In this case, the authors calculated the coupling energies between amino

AXON GROWTH

Fas track to recovery

The regeneration of axons after peripheral nerve injury depends on the activation of signalling pathways that induce neurite sprouting from the damaged neurons. In *Nature Cell Biology*, Desbarats *et al.* show that a pivotal component of one such pathway is the Fas receptor, which is more commonly associated with cell death, and they also provide some intriguing insights into the molecular mechanisms that underlie Fas-mediated neurite growth.

Fas was initially identified in lymphocytes, in which crosslinking of Fas receptors by the Fas ligand (FasL) stimulates the cleavage of caspase 8. This initiates the caspase cascade, which culminates in apoptotic cell death. However, it is becoming increasingly clear that in other cellular contexts, including some neurons and glia, the engagement of Fas receptors by FasL or Fas-specific antibodies can have the opposite effect of stimulating growth.

Desbarats *et al.* examined the consequences of Fas engagement in two cell lines — a T-cell leukaemia line and a neuroblastoma line. They found that in the T cells, Fas engagement activated the caspase cascade, and the cells underwent apoptosis. In the neuroblastoma line, the outcome was different — caspase 8 was not cleaved and the cells did not die. Instead, the cells responded normally to the culture conditions by extending fine neurites, and they activated the extracellular-signal regulated kinase (ERK) cascade, which has previously been implicated in neurite outgrowth. By treating dorsal root ganglion explants with Fas crosslinking antibodies, the authors showed that Fas-mediated activation of the ERK cascade can promote neurite growth in primary sensory neurons.

Crucially, Desbarats *et al.* showed that Fas engagement also stimulates axon regeneration *in vivo*. In mice, they measured the time taken for functional recovery of a limb after a sciatic nerve crush injury, and they found that this process was delayed in a Fas-deficient mutant line. Conversely, injecting a Fas crosslinking antibody at the site of injury in wild-type mice accelerated their recovery. A mutation that only affected the death domain of the Fas receptor did not adversely affect functional recovery, implying that the pro-apoptotic activity of Fas is not required for axon regeneration.

It is still not clear what factors cause Fas to activate the ERK pathway rather than the caspase cascade, although it seems likely that the outcome of Fas engagement depends not only on the cell type, but also on the metabolic state of the cell. It is also interesting to note that the neurons that Desbarats *et al.* used in this study expressed very low levels of caspase 8. If the conditions that favour the activation of the ERK pathway over the caspase cascade can be identified, it might be possible to use this knowledge to develop new therapeutic approaches to treat nerve injury.

Heather Wood

References and links

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FURTHER READING Wajant, H. The Fas signaling pathway: more than a paradigm. *Science* **296**, 1635–1636 (2002)

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

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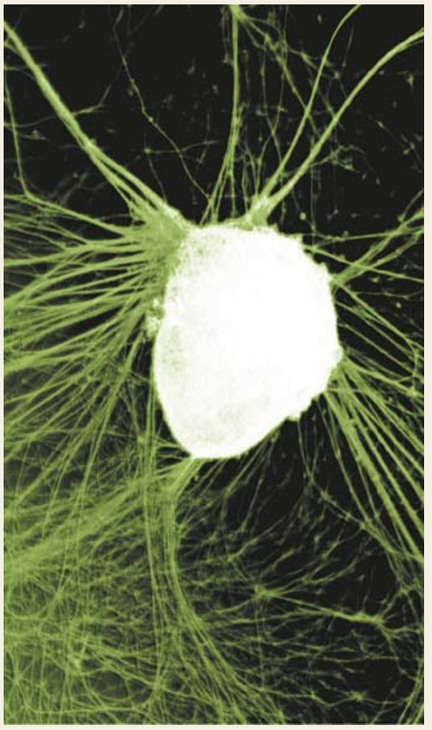
ORIGINAL RESEARCH PAPER Kash, T. L. *et al.* Coupling of agonist binding to channel gating in the $GABA_A$ receptor. *Nature* **421**, 272–275 (2003)



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

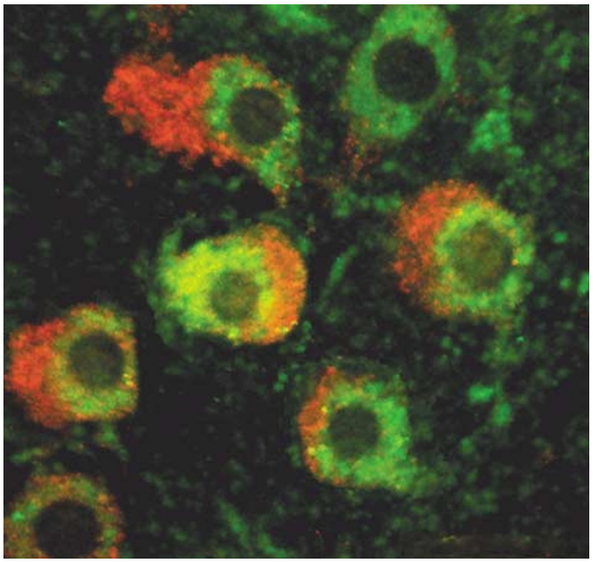
Activation of caspase 3 has become almost synonymous with cell death. So it comes as a surprise to discover that caspase 3 cleavage seems to be an essential part of a neuroprotective effect known as preconditioning. Experiments described by McLaughlin *et al.* in *Proceedings of the National Academy 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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