NEUROLOGICAL DISORDERS

Striking point for stroke

Glutamate toxicity as a result of excessive NMDA (*N*-methyl-D-aspartate) receptor activation is considered the main factor responsible for neuronal death following ischaemia. However, the essential physiological action of these receptors in synaptic transmission means that simply blocking them is not a feasible therapeutic option. In a study published in *Cell*, the authors uncover a specific cell death signalling protein, death-associated protein kinase 1 (DAPK1), that interacts with the NMDA receptor subunit NR2B to induce a deadly Ca^{2+} influx. Uncoupling this interaction drastically reduces stroke damage in mice.

NMDA receptor activation requires the binding of both glutamate and glycine, as well as membrane depolarization, to allow the influx of Ca^{2+} through the channel pore. Under physiological conditions, the influx of Ca^{2+} partially inhibits the receptor to prevent Ca^{2+} overload, but it is unclear how this negative feedback mechanism is disabled in stroke.

DIGITAL VISION

The authors found that DAPK1 immunoprecipitated with the NMDA receptor complex in cortical extracts from mice subjected to focal cerebral ischaemia. Moreover, they showed that DAPK1 directly binds to the NMDA receptor subunit NR2B and that a synthetic peptide comprising NR2B amino acids 1292–1304 ($NR2B_{CT}$) prevented NR2B–DAPK1 binding without affecting the association of postsynaptic density protein 95 or Ca^{2+}–calmodulin-dependent protein kinase type 2 with the receptor subunit. Experiments in which recombinant and mutant NR1–NR2B receptors were co-expressed with constitutively active DAPK1 in HEK293 cells indicated that activation of DAPK1 increases NMDA channel conductance by phosphorylating the NR2B subunit at Ser1303.

As overall synaptic protein composition and NMDA receptor physiology were not affected in mice lacking the *Dapk1* gene, the authors assessed the functional effects of DAPK1 deletion on NMDA receptor toxicity in two mouse models of ischaemia. After transient global ischaemia by carotid artery occlusion for 20 minutes, most of the neurons in the hippocampus, layer 3 of the cortex and the striatum had degenerated in wild-type mice. Interestingly, markedly less damage was observed in the *Dapk1*$^{-/-}$ mice. In a model of focal cerebral ischaemia by middle cerebral artery occlusion for 90 minutes, the infarct volume was reduced in the knockout mice, and this was associated with an improvement of neurological scores.

To examine the therapeutic implications of these findings, the authors tested the effect of a cell membrane-permeant $NR2B_{CT}$ peptide, which uncouples activated DAPK1 from NR2B in a mouse model of stroke. Although DAPK1 was activated, its association with NR2B, NR2B phosphorylation at Ser1303 and the increase in Ca^{2+} influx through NMDA receptors was blocked. Administration of the peptide as long as 1 hour after stroke onset both reduced the total infarction volume and improved neurological functions, suggesting a potential new strategy for the treatment of stroke.

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ORIGINAL RESEARCH PAPER Tu, W. *et al.* DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. *Cell* **140**, 222–234 (2010)