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COMMENTARY

Pre-conditioning protection in the brain

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It was with cardiac preparations that the discovery was made that brief periods of ischaemia could reduce or prevent substantial damage occasioned by a subsequent, more prolonged ischaemic episode (Murry *et al*., 1986). The phenomenon, now known as preconditioning, has received most attention in the cardiac field because of its potential therapeutic importance. Small repeated degrees of mild ischaemia induced by exercise or artificial high-rate pacing, could precondition the heart to resist later major insults. Further, if the mediators or mechanisms involved in pre-conditioning could be determined, it might be possible to reproduce the protection using pharmacological agents in patients in whom exercise or pacing might be impractical or unsafe options.

It is now recognised that a similar phenomenon can be induced in the central nervous system (CNS). Neuronal ischaemic preconditioning can be demonstrated *in vivo* (Kitagawa *et al*., 1990), in brain slices (Schurr *et al*., 1986) and in cultures (Khaspekov *et al*., 1998). The paper by Pugliese *et al*. (2003) explores two aspects of this phenomenon. Firstly, they have concentrated, not on the simple hypoxic models used in most previous studies of preconditioning *in vitro*, but on the less-studied low oxygen–low glucose model (oxygen–glucose deprivation, OGD), which the authors consider closer to the conditions that would accompany a cerebral infarct *in vivo*. The use of a term such as OGD is strongly to be recommended in a work such as this. Apart from the scientific advantage that it describes more accurately the experimental conditions used, the term 'ischaemic' makes little sense in an *in vitro* experiment, and could be misleading.

Of course, even OGD could prove to be one of those experimental modifications which, because it resembles the pathological situation more closely than simple hypoxia, blinds us to its limitations and the recognition that it is, after all, only a model. In reality, the generation of ischaemia will presumably be accompanied by a plethora of changes within the blood (release of amines, eicosanoids, growth factors, cytokines), and the biochemical consequences of interactions between those substances and similar compounds released by vascular endothelial cells, neurones and glia. Indeed, the failure of glutamate receptor antagonists to prevent ischaemic damage in human stroke patients is probably due to the occurrence of such a myriad of chemical complications occurring in an infinite variety of temporal and geographical patterns, which no animal model can mimic.

The second feature of the paper by Pugliese *et al*. (2003) is a detailed examination of the role of adenosine receptors. Adenosine has been recognised as a cardioprotective agent for many years, contributing to preconditioning at least partly by activating K_{ATP} channels (Yao & Gross, 1994). Pugliese *et al*. (2003) confirm previous evidence that A_1 receptor blockers prevent the suppression of neuronal activity induced by OGD, and that they prevent completely the preconditioning protective effect of brief OGD periods on a subsequent major insult.

Perhaps surprisingly, A_{2A} receptors do not seem to be involved in OGD preconditioning. It might be anticipated that the production of high concentrations of extracellular adenosine would activate A_{2A} receptors and facilitate glutamate release, especially since A_{2A} receptors suppress the inhibitory effects of activating A_1 receptors (O'Kane & Stone, 2000). The lack of involvement of A_{2A} receptors is also surprising, given that A_{2A} receptor antagonists are neuroprotective (Phillis, 1995; Jones *et al*., 1998). However, Pugliese *et al*. (2003) report that A_{2A} receptor blockers did not modify slice preconditioning. It may be that the resolution of this difference lies in the preparation, since the protective effect of antagonists and an injurious effect of agonists (Melani *et al*., 2003) were observed *in vivo*, not in slices.

Antagonists for A_3 adenosine receptors, however, facilitated the post-OGD recovery of hippocampal potentials after a prolonged insult, producing a better recovery than preconditioning alone. The A_3 receptor population has recently become easier to study with the availability of selective antagonists, and these data are entirely consistent with earlier work which suggested that the acute activation of A_3 receptors exacerbated ischaemic damage *in vivo*. Indeed, the implication is that A_3 receptor blockers could be among the most protective compounds yet tested against ischaemic damage in the brain.

Unfortunately, this paper brings us no closer to understanding the specific mechanisms of protection by adenosine. It is possible that entirely different mechanisms operate in the heart and brain, but it is unlikely that alteration of glutamate release or receptor activation are relevant in the former, or that changes in the release of eicosanoids or cytokines are relevant in the brain, at least on the time course of these experiments.

Neither do we yet understand the relationship between adenosine and amino acids in preconditioning. Adenosine can block the activation of NMDA receptors (Norenberg *et al*.,

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1997), while NMDA receptors can reduce the inhibitory effects of adenosine (Nikbakht & Stone, 2001) apparently by facilitating the activation of excitatory A_{2A} receptors. Antagonists of NMDA can reduce preconditioning *in vivo* (Bond *et al*., 1999), but is this due to NMDA receptor blockade directly, or due to a blockade of the adenosine release which NMDA receptor activation produces?

A very interesting avenue of research would be to explore the relationships between ischaemic preconditioning and related paradigms in which a chemical stimulus is used to induce tolerance or protection. It was shown in 1986 that the loss of neuronal activity and synaptic transmission produced by cortical applications of NMDA, *in vivo*, recovered spontaneously during maintained applications, and no further response to NMDA could be obtained for about an hour (Addae & Stone, 1986). Similar protective interactions can be demonstrated between NMDA and AMPA applications (Addae *et al*., 2000), neither being mediated by adenosine. In a further development of this work, it has now been shown that induction of hippocampal long-term potentiation (LTP) prevents the suppression of synaptic transmission by hypoxia (Youssef *et al*., 2001). This protection is not mediated by nitric oxide, and implies that the activation of NMDA receptors semiphysiologically can 'precondition' the neuronal networks to resist a subsequent NMDA application or hypoxic insult.

Many fascinating questions remain in this area. Does preconditioning alter neuronal excitability to NMDA or other glutamate agonists? Does it lead to changes in adenosine or glutamate release? Does it modify the interactions between adenosine and glutamate receptors? Could repeated strenuous exercise produce a sufficient degree of mild cerebral ischaemia to afford even a small degree of preconditioning protection against stroke? Does chronic smoking produce periods of mild cerebral ischaemia which could contribute to the anecdotal protective action of smoking against vascular and Alzheimer's dementias? And does our chronic consumption of dietary xanthines, by blocking and/or upregulating adenosine receptors, increase our susceptibility to stroke or compromise our ability to benefit from preconditoning for those lucky enough to survive a first episode? And most importantly of all – can we mimic preconditioning pharmacologically and prevent the awful tragedies which strokes can cause?

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