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BRAIN RESEARCH

Research Report

Preconditioning with 4-aminopyridine protects cerebellar granule neurons against excitotoxicity

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ABSTRACT

Preconditioning by excitatory stimuli such as *N*-methyl-D-aspartate (NMDA) offers good neuroprotection against excitotoxic insults, but is potentially limited by the risk of damage associated with the treatment. We report here the potential of an alternative strategy, tested on rat neonatal cerebellar granule neurons, which involves a 48-hour preconditioning step using the potassium channel blocker 4-aminopyridine (4-AP), at a low (50 μM) and at a higher (2500 μM) concentration (in the presence or absence of the $GABA_A$ receptor antagonist, bicuculline). 4-Aminopyridine gave extensive protection against a number of stressors (glutamate, NMDA and 3-nitropropionic acid) applied 24 h following the end of the preconditioning period. Blockade of neuronal depolarisation by tetrodotoxin during preconditioning attenuated but did not eliminate protection, whilst co-application with the NMDA receptor blocker MK-801 increased protection. Western blot analysis showed that CREB phosphorylation was significantly increased by the 4-AP preconditioning, although bcl-2 expression was not stimulated. Glutamate induced cell death without significant activation of caspase-3, suggesting that 4-AP preconditioning is effective primarily against necrotic excitotoxicity. Since 4-AP preconditioning affords extensive protection against a range of neurotoxic insults we propose that it could provide the basis for a novel neuroprotective therapy worthy of further investigation.

1. Introduction

The term preconditioning refers to the protection, against a range of otherwise damaging insults, which can be afforded by prior exposure to mild, innocuous or sublethal conditions. Although it was first described in cardiac tissue (Murry et al., 1986), preconditioning is now recognised to occur also in the CNS, where it has been demonstrated *in vivo* (Kato et al., 1991; Liu et al., 1992; Miyashita et al., 1994; Blondeau et al., 2000), and *in vitro* using models of anoxia, hypoxia, ischaemia and oxygen–glucose deprivation (Schurr et al., 1986; Centeno et al., 1999; Pugliese et al., 2003). Subtoxic excitatory stimulation has been used to precondition neurons against subsequent excitotoxic insults (Kitagawa et al., 1990; Tauskela et al., 2001; Meller et al., 2005; Smith et al., 2008).

The severity of the preconditioning stage required to achieve effective protection may however prove to be a drawback that limits potential application (Dirnagl et al., 2003). An

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alternative preconditioning approach to induce neuronal protection has recently been examined, using a more prolonged, but lower intensity, stimulation of glutamate receptors, prior to the neurotoxic stimulus (Papadia et al., 2005, 2008; Soriano et al., 2006; Tauskela et al., 2008). In rat hippocampal neurons, Soriano et al. (2006) attempted stimulation of

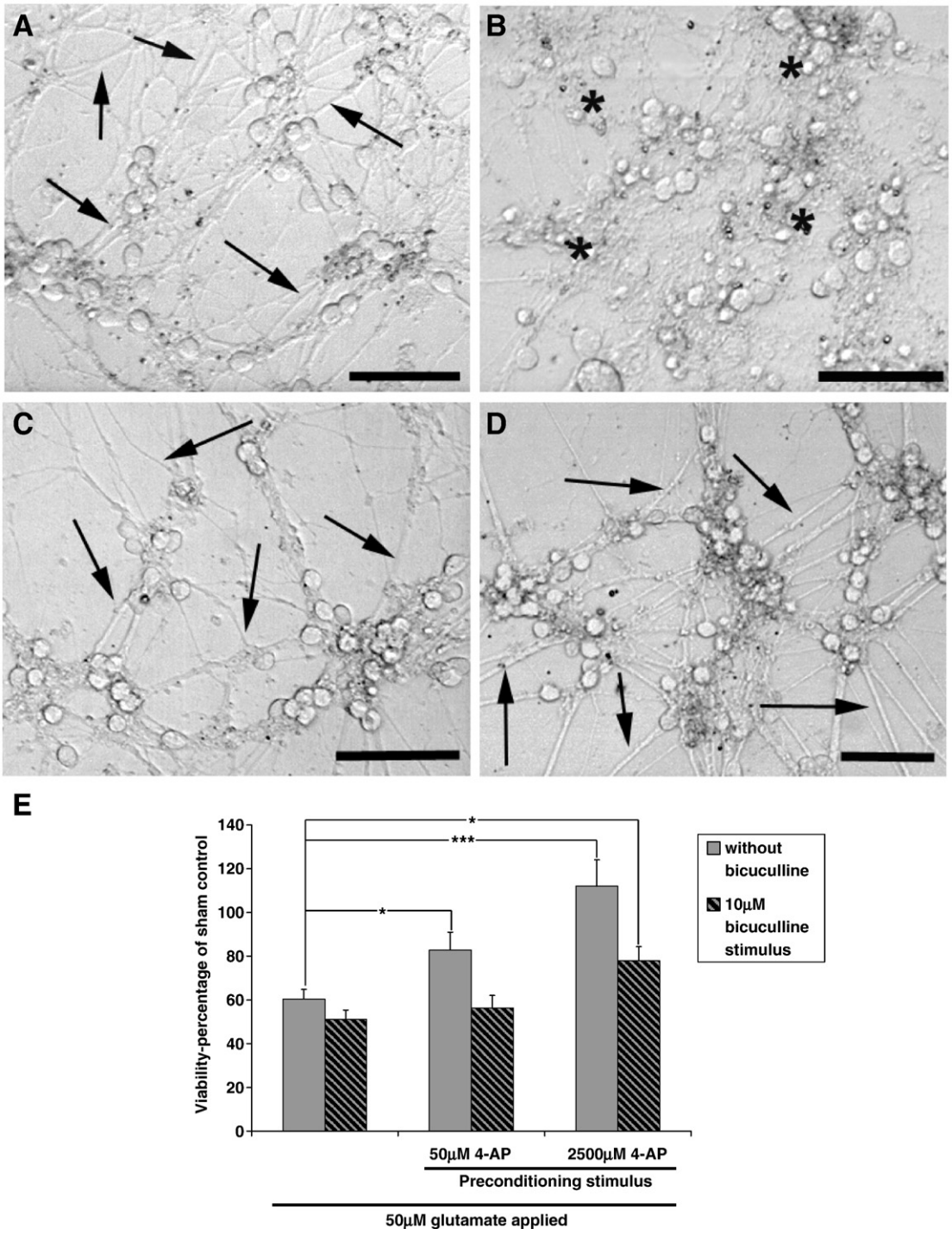

Fig. 1 – (A) Control morphology of untreated CGNs highlighting the good neuritic outgrowth (arrows) which typically extends from the rounded perikarya. (B) Exposure to 50 μM glutamate for 24 h resulted in reduced numbers of CGNs, with damage to remaining cells evidenced by shrunken perikarya (*) and a loss of neurites compared to the control morphology. (C) Control neuronal phenotypes were retained following addition of 2500 μM 4-AP for 48 h. (D) CGNs preconditioned with 2500 μM 4-AP prior to 24 h 50 μM glutamate resembled the untreated control phenotype and not one associated with a glutamate insult. Bars=50 μm. (E) The effects on cell viability (assayed with fluorescein diacetate) of a 24 h treatment with 50 μM glutamate and the protection afforded by preconditioning with 50 or 2500 μM 4-AP (for 48 h) prior to treatment 24 h later. Also shown are the effects of preconditioning with 50 or 2500 μM 4-AP in the presence of 10 μM bicuculline. The reduction in protection seen with bicuculline co-application was not significant for any treatment group. Mean±S.E.M. ($n=5$) $^{*}p<0.05$, $^{***}p<0.001$.