CALORIC-RESTRICTION AND DOCOSAHEXAENOIC ACID ARE NEUROPROTECTIVE IN AN IN VITRO MODEL OF MECHANICAL STRESS

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To characterize the effect of traumatic injury on neurones and glia, and to evaluate, in a first screen, potential neuroprotective agents, we set up an in vitro model of mechanical injury in dorsal root ganglion (DRG) cells. Then, to validate the predictive value of this model, we investigated four factors known either to influence the intrinsic response to injury or to confer neuroprotection. These are: animal strain (Schmitt et al., 2006), age (Gwak et al., 2004), calorie restriction (Hiona and Leeuwenburgh 2004) and treatment with the omega-3 fatty acid docosahexaenoic acid (DHA) (King et al., 2006). DRG cultures were prepared as described (Gavazzi et al., 1999) from female rats sacrificed by CO₂ inhalation. For the strain comparison we used adult (3-6 months; 230-270 g) Wistar and Sprague-Dawley rats. For the age comparison we used adult and aged (21-24 months; 600-700 g) Sprague-Dawley rats. For the calorie restriction we used aged Sprague-Dawley rats fed either ad libitum or a 45% calorie restricted-diet (21-24 months; 450-600 g) from 6 months of age. DRG cells were plated onto the elastic membranes of Flex I® culture plates (Dunnlab). After 48 h, cultures were subjected to 1 h of nominal 29% static stretch or no stretch using a FX-2000™ baseplate (Flexcell Int.). Some cultures were exposed to DHA (1 µM) either during the stretch or for 24 h following the stretch. After a further 24 h, cells were labelled with the cell injury marker ethidium homodimer-1 (EthD-1, 2 µM, Invitrogen), the neurone-specific marker β-tubulin III (1:1000, Sigma) and bisbenzimide (Hoechst 33342, 2 µg/mL, Sigma). Tissue in each experimental condition originated from 4 – 12 rats. The number of EthD-1 positive cells was counted in 8 regions per well and 3 wells were used per condition. Data was expressed as means ± S.E.M. and analysed with ANOVA. Stretch of Wistar adult rat cultures significantly increased the number of injured neurones; 9 ± 1% to 23 ± 2% (p<0.05). Stretch did not injure non-neuronal cells. Similar neuronal injury occurred in stretched DRG neurones from adult or aged Sprague-Dawley rats. In contrast, stretch did not injure neurones from calorie-restricted aged rats; neuronal injury was 5 ± 1% and 6 ± 1% in un-stretched and stretched cultures, respectively. Concomitant or post-stretch treatment with DHA also totally prevented the stretch-induced neuronal injury. To conclude, our in vitro model of mechanical stress injures neuronal but not non-neuronal cells. The injury was mild, in terms of percentage of affected cells, and not influenced by animal strain or age. In contrast, tissue from calorie-restricted aged rats or adult rats treated with DHA showed a total reversal of stretch-induced neuronal damage. These data support the in vivo neuroprotective potential of omega-3 fatty acids and highlight the potential of calorie restriction in mechanically-induced neuronal injury.


This work was supported by the Corporate Action Trust.