Mild Concussive Head Injury Results in Increased Brain Substance P Immunoreactivity

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The neuroinflammatory neuropeptide substance P (SP) has been implicated in oedema development following traumatic brain injury. Whether the neuropeptide plays a role in concussive head injury is unknown. Accordingly, we have used a newly developed model of mild head injury in rats to characterise the release of SP following concussive brain injury. Following brain trauma, there was no evidence of motor or cognitive deficits over the ensuing 3 weeks. Despite this, increased SP immunoreactivity was present in perivascular axons, some pyramidal neurones and astrocytes when compared to sham animals. Our findings suggest that concussive brain injury predisposes an individual to diffuse brain swelling, which may have implications in the management of sports related concussion.

Introduction

Concussion is a mild form of traumatic brain injury (TBI) that by definition incorporates immediate and transient impairment of neurological function that may include alterations of consciousness, and disturbances of vision and/or equilibrium, amongst others (Committee of Head Injury, 1966). In sporting events, mild head injury is common, and without overt signs such as loss of consciousness, the concussion may go untreated because few symptoms may be visible to the casual observer (Maddocks et al, 1995). The danger in this is that a second concussive event within a short period of time may lead to a condition that has been termed ‘second impact syndrome’. While not universally accepted as a

syndrome in itself (McCrory, 2001), it is widely recognized that young athletes may exhibit diffuse cerebral swelling as a complication of diffuse TBI, and that this may be exacerbated while symptomatic from an earlier concussive event (Cantu, 1998; Saunders & Harbaugh, 1984).

The mechanisms associated with the development of diffuse cerebral swelling following mild TBI still remains unclear. We have recently shown in an experimental model of severe, diffuse TBI that neuropeptide release leads to an opening of the blood brain barrier and profound oedema formation (Nimmo et al., 2004). Moreover, the neuropeptide responsible for the increased vascular permeability and subsequent oedema formation was identified as substance P, and inhibition of its actions prevented oedema formation and resulted in a highly significant improvement in post-traumatic neurological outcome. However, it is not always possible to extrapolate results from severe TBI to a condition of mild TBI, given the absence of many of the neuropathological changes at the lower injury severity. Accordingly, the aim of the present study was to identify whether the neuroinflammatory neuropeptide, substance P, was released following a mild (concussive) level of head injury.

Materials and Methods

All experimental protocols were approved and conducted according to the guidelines established for the use of animals in experimental research as outlined by the Australian National Health and Medical Research Council.

Adult male Sprague–Dawley rats (n=24; 400 ± 25 g) were subjected to a mild TBI using the newly developed Cernak model of impact acceleration brain injury (Cernak et al., 2004). Briefly, animals were anaesthetised with halothane, the skull exposed with a midline incision, and a stainless steel disc (10 X 3 mm) fixed centrally on the exposed skull between lambda and bregma using polyacrylamide adhesive. The animal was then placed vertically beneath an accelerating metal impactor and injury induced by initiating contact between the impactor and the metal disc fixed to the animal's skull. The distance the impactor travelled after contacting the disc was restricted to less than 15 mm, equating to a mild TBI. A further 15 animals were used as sham (surgery but no impact) controls.

After injury, motor and cognitive outcome (n=6/group) was assessed using the rotarod and Barnes Maze tests, respectively, as previously described in detail elsewhere (Nimmo et al., 2004). Briefly, the rotarod test requires an animal to walk on a motorised rotating assembly of 18 rods for up to 2 min as the rotational speed of the assembly is increased from 0 to 30 revolutions per minute. The duration in seconds at which the animal completes the task or falls from the rods is recorded. The Barnes maze requires the animal to be placed under a cover in the centre of an elevated 1.2-m diameter platform containing 19 holes around the periph-

ery. One of the holes is the entrance to a darkened escape tunnel and is not visible from the surface of the platform. After activating a series of bright lights and an aversive sound, the cover is lifted and the latency in seconds for the animal to locate and enter the darkened escape tunnel is recorded.

For immunohistochemical analyses, injured and sham animals were anaesthetised with halothane at 3 days or 5 days post-trauma (n = 3/time point), and were perfused transcardially with 4% paraformaldehyde for 1 to 2 min. The brains were then removed and coronal sections (3–5 mm) cut and embedded in paraffin. Serial sections (5 μm) were cut from approximately – 4.0 mm relative to bregma using a Leitz rotary microtome (Leica, Malvern, PA) and mounted on poly-L-lysine coated slides for staining with haematoxylin and eosin (H&E), APP (monoclonal antibody 22C11) or substance P.

Results

As expected at this mild level of TBI, no injured animal displayed any motor or cognitive deficits as compared to sham animals (results not shown). This was consistent with the absence of any axonal injury at any time point as identified by APP immunostaining, and the lack of overt cell death in H&E stained sections. Minor and transient dark cell change was apparent in the hippocampus at 3 days post-trauma, but no significant changes were observed in the cortex at any time point. These changes are consistent with a concussive level of injury that may be accompanied by a mild and transient cognitive deficit that remained undetected in our tests.

In contrast to the absence of any significant neuropathological or neurological changes after injury, all injured animals demonstrated a profound increase in substance P immunoreactivity (Fig. 1). This was particularly

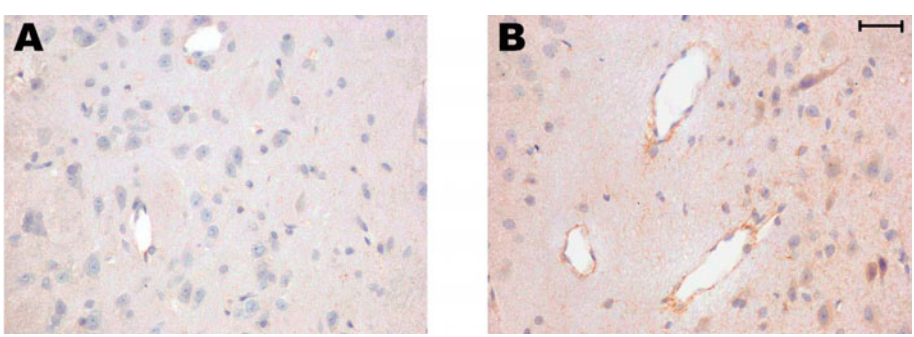

Figure 1. *Substance P immunoreactivity in shams (A) and at 3 days following mild concussive traumatic brain injury (B) in rats. Note the darkly stained substance P positive axons around the cortical vessels and the presence of substance P positive neurones after injury (B). Bar = 100μm.*

apparent in the cortex within cortical perivascular axons, some pyramidal neurones and in astrocytes. There were also substance P positive neurones in the hippocampus.

Conclusions

Increased substance P immunoreactivity after mild TBI was apparent in the absence of any overt functional deficits (motor or cognitive) or any significant neuropathological changes. Because substance P is one of the main neuropeptides involved in the development of neurogenic inflammation (Campos and Calixto, 2000), our results suggest that even mild TBI may lead to the development of diffuse cerebral swelling. It remains to be determined if a second impact of an injured brain that is undergoing substance P release exacerbates the resultant injury.

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