018P IDENTIFICATION OF THE NMDA RECEPTOR SUBTYPE INVOLVED IN THE DEVELOPMENT OF DISEASE DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the human central nervous system (CNS) that is characterised by episodes of blood-brain barrier (BBB) breakdown with associated oedema and neuroinflammation. The mechanisms contributing to BBB dysfunction during MS and its counterpart experimental autoimmune encephalomyelitis (EAE) are not fully understood, but a role for the *N*-methyl D aspartate (NMDA) receptor has been recently indicated. Our studies have demonstrated NMDA receptor antagonism to suppress BBB dysfunction in acute EAE concurrent with a reduction in neurological signs and neuroinflammation (Paul & Bolton, 2002). However, the subtype of the NMDA receptor population involved is unknown. NMDA receptors are composed of an NR1 subunit that is essential for function, and one or more NR2 subunits, A-D, which dictates receptor subtype. The aim of the current study was to identify the NMDA receptor subtype(s) potentially involved in EAE and therefore BBB dysfunction.

CNS levels of NMDA receptor subunits NR1, NR2A and NR2B were examined using Western blotting. Cerebellum, medulla-pons and cervical spinal cord tissues were examined from individual male Lewis rats (225-250g at inoculation) selected from: normal; complete Freund's adjuvant control; day 13 post-inoculation (p.i.) for EAE [height of disease]; and day 21 p.i. for EAE [early recovery from disease] Three profiles for each CNS region were studied (n=3) in duplicate.

NR1 tissue profiles indicated no consistent alteration in the pattern of NR1 subunit expression. However, an increase in NR2A subunit expression during the height of disease was clear showing typically 0.7, 0.5 and 6 fold increases in cerebellum, medulla-pons and cervical spinal cord respectively, compared to normal animals. In addition, the NR2B subunit also demonstrated a subtle elevation in all regions at day 13 p.i. (typically 0.5, 0.3 and 0.2 fold in cerebellum, medulla and cervical spinal cord respectively compared to normal controls). Interestingly, further changes in detected NR2A subunits occurred during early recovery. In particular, NR2A subunit levels were further enhanced at day 21 p.i. in the cervical spinal cord (up to 8 fold greater than normal values), while the cerebellum was reproducibly depressed compared to day 13 p.i. showing a 2-4 fold decrease compared to normal control in 2 out of 3 samples. This last observation reflects the continuing yet altered pathological state of the EAE animal during the early recovery phase previously reported in BBB studies (Paul & Bolton., 1994).

The research presented provides further evidence for NMDA receptor involvement in EAE, in particular receptors containing the NR2A subunit. Identification of specific receptor subtypes involved in disease may offer novel therapeutic targets for the future management of MS.

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