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Aging, cortical injury and Alzheimer's disease-like pathology in the guinea pig brain

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized histopathologically by the abnormal deposition of the proteins amyloid-beta (Aβ) and tau. A major issue for AD research is the lack of an animal model that accurately replicates the human disease, thus making it difficult to investigate potential risk factors for AD such as head injury. Furthermore, as age remains the strongest risk factor for most of the AD cases, transgenic models in which mutant human genes are expressed throughout the life span of the animal provide only limited insight into age-related factors in disease development. Guinea pigs (*Cavia porcellus*) are of interest in AD research because they have a similar Aβ sequence to humans and thus may present a useful non-transgenic animal model of AD. Brains from guinea pigs aged 3–48 months were examined to determine the presence of age-associated AD-like pathology. In addition, fluid percussion-induced brain injury was performed to characterize mechanisms underlying the association between AD risk and head injury. No statistically significant changes were detected in the overall response to aging, although we did observe some region-specific changes. Diffuse deposits of Aβ were found in the hippocampal region of the oldest animals and alterations in amyloid precursor protein processing and tau immunoreactivity were observed with age. Brain injury resulted in a strong and sustained increase in amyloid precursor protein and tau immunoreactivity without Aβ deposition, over 7 days. Guinea pigs may therefore provide a useful model for investigating the influence of environmental and non-genetic risk factors on the pathogenesis of AD.

1. Introduction

The most widely accepted hypothesis addressing the pathologic basis of Alzheimer's disease (AD) is the "amyloid hypothesis", which suggests that the altered metabolism of a small peptide, amyloid-beta (Aβ), is the trigger for the pathogenic cascade that ultimately results in neuronal dysfunction and death. Aβ is derived through sequential enzymatic cleavage of the amyloid precursor protein (APP), a type 1 integral membrane protein, by β-site APP cleaving enzyme (BACE) and then γ-secretase, a multi-subunit enzymatic complex. Age is the strongest risk factor that has been identified to date, yet it is still unclear as to why the clinical symptoms of AD appear in later life when the neuropathology may begin many years, even decades, earlier (Villemagne et al., 2013).

A major hindrance to AD research is the lack of an animal model that replicates all the features of the human disease, namely a chronic, age-dependant neurodegenerative disease with characteristic AD histopathology. Transgenic animal models have yielded a number of important insights into disease mechanisms and are a useful tool for therapeutic design and evaluation. However, the genetic manipulation necessary to produce them does not accurately reflect the human disease state, and accordingly limits at least some of the conclusions that can be drawn.

An alternative strategy may be to investigate potential risk factors in species with similar Aβ sequence to humans. One candidate is the guinea pig (Beck et al., 1997). In vitro studies using guinea pig neuronal cells have demonstrated that APP processing in this species is identical to that in humans (Beck et al., 2000, 2003). Guinea pigs have been used in a small number of studies to investigate the effect of glutamate (Stephenson and Clemens, 1998), serotonin

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(Arjona et al., 2002), estrogen (Petanceska et al., 2000), testosterone (Wahjoepramono et al., 2008, 2011), β-secretase inhibitors (Hook et al., 2007; Jeppsson et al., 2012), and cholesterol (Fassbender et al., 2001) on Aβ production. Further, the effect of protein kinase C (PKC) (Rossner et al., 2000) and gamma amino butyric acid ($GABA_A$) receptor modulation (Marcade et al., 2008) on APP processing has also been investigated in this species.

Traumatic brain injury (TBI) has been identified as a risk factor for AD (Bachman et al., 2003; Fleminger et al., 2003). Following TBI, cerebrospinal fluid levels of tau (Franz et al., 2003), APP and Aβ increase (Olsson et al., 2004) and *postmortem* analyses of brains from TBI patients show widespread distribution of Aβ containing plaques and other AD-like pathology including gliosis (Gentleman et al., 1993; Griffin et al., 1994; Horsburgh et al., 2000; Ikonomovic et al., 2004). In mice (Iwata et al., 2002), rats (Blasko et al., 2004), and sheep (Van den Heuvel et al., 1999), APP messenger RNA and protein expression rapidly, albeit transiently, increase in response to cortical injury.

To date, no studies have investigated the relationship between aging and AD-like pathology in a non-primate species with human-like APP. Similarly, head injury models have not been performed in animals with similar Aβ sequence to humans. Therefore, a series of pilot studies was conducted to investigate the suitability of the guinea pig as a non-transgenic animal model for AD. A longitudinal aging study and a fluid percussion-induced brain injury (FPI) study were conducted to determine whether any AD-like pathology could be detected in the guinea pig brain.

2. Methods

All animal experimentation was approved by the Animal Ethics Committees of The University of Western Australia and the Institute of Medical and Veterinary Sciences and carried out under published National Health and Medical Research Council guidelines. Tissue collection was performed after a lethal dose of sodium pentobarbitone followed by transcardial perfusion with fixative (10% buffered formal saline) or by harvesting fresh unfixed tissue snap frozen in liquid nitrogen. Tissue embedding and preparation for histochemical and western blotting procedures were performed as previously described (Bates et al., 2002, 2007).

The following antibodies were used to investigate APP and Aβ metabolism: WO2 (gift from Professor Colin Masters, University of Melbourne, VIC Australia, 1/10 for histochemistry, 1/100 for western blots), anti-BACE1 polyclonal antibody (Thermo Scientific, IL, USA, 1/100 for histochemistry), and anti-nicastrin polyclonal antibody (Nct, Invitrogen, CA, USA, 1/100 for histochemistry). In addition, tau was investigated using a monoclonal Tau-5 antibody (Invitrogen, CA, USA, 1/1000 for histochemistry, 1/5000 for western blots). A modified Congo red stain (Bancroft and Stevens, 1982) was used to detect the presence of fibrillar Aβ deposits. Astrocyte reactivity was investigated using monoclonal anti-GFAP antibody (Sigma, MO, USA, 1/400 for histochemistry, 1/5000 for western blots).

2.1. Aging study

Animals at the age of 3, 6, 9, 12, 18, 24, 36, and 48 months of both sexes were used. Each age group had 7 animals, 3 were processed for histologic examination and the remaining 4 brains were dissected into 10 brain regions; frontal, parietal, occipital and temporal cortices, cerebellum, hippocampus, thalamus, midbrain, brainstem, and basal ganglia for western and enzyme linked immunosorbent assay (ELISA) analysis (see in the following).

2.2. Fluid percussion injury study

Animals were fasted (with access to water) 12 hours before surgery to assist in recovery from anesthesia. Anesthesia was achieved as follows; 0.1 mL atropine sulfate (0.6 mg/mL) was administered subcutaneously. After 5 minutes, diazepam (5 mg/kg intraperitoneally, Parnell Laboratories, Sydney, NSW, Australia) was given intraperitoneally at a dose of 5 mg/kg body weight. Once the animal was sufficiently sedated (approximately 20 minutes post diazepam injection) hypnorm was given intramuscularly in the rear limb (VetaPharm, 0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone, 1 mL/kg intramuscularly).

A mid-sagittal incision was made and a 5-mm craniotomy performed centered over the left parietal cortex (Dudas et al., 2000) to facilitate induction of lateral FPI as previously described (Vink et al., 1988). The dura was left intact and a female leur-loc was placed over the craniotomy and secured with dental cement. The fluid percussion device consisted of a plexiglass cylindrical reservoir, 60 cm long and 4.5 cm in diameter, bounded at one end by a plexiglass, rubber-covered piston mounted on O-rings. The opposite end was fitted with a 2 cm long metal housing on which a pressure transducer was mounted and connected to a 5 mm tube (2 mm inner diameter) that terminated with a male leur-loc fitting. The whole system was filled with physiological saline at room temperature.

At the time of injury the male leur-loc tube was connected securely to the female leur-loc on the skull. To produce an insult, a 4.8 kg pendulum was dropped from a pre-determined height to impound the rubber-covered piston, producing a pressure pulse of 2.1 ± 0.2 atmospheres. We have previously shown that this equates to a mild to moderate injury in rats (Vink et al., 1988). Injury resulted in apnea so animals were manually resuscitated for a period ranging from 2 to 15 minutes. The scalp was then closed with surgical staples. One dose of postoperative analgesia (0.16 mg/kg buprenorphine intraperitoneally) along with 1 mL sterile saline/hour for 4 hours was administered. Sham animals underwent the same procedures without the FPI. From the moment of sedation to recovery, animal temperature was maintained throughout using a thermostatically controlled heating blanket.

2.3. Protein detection and analysis: western blotting and ELISA

The method for tissue preparation and western blot analysis has been described previously (Bates et al., 2002, 2007; Martins et al., 2001). Protein concentration in the sample homogenate was calculated using the Micro BCA protein assay kit (Pierce, IL, USA). Samples containing 25 μg of total protein were resuspended in sample buffer (166 mM Tris-HCl, 8% SDS, 0.53 M glycine, 5% β-mercaptoethanol, pH 6.8). Samples were arranged such that each gel contained samples from each age group for a particular brain region. An internal control sample was created by pooling cerebellum homogenates from different ages and this was run on each gel to account for gel-to-gel variations. Cerebellum was chosen because this was the easiest brain region to distinguish, is usually spared from AD-like pathology in human disease, and yielded more sample than required for the experiments.

After electrophoretic separation and transfer of proteins, the nitrocellulose membranes were cut into sections corresponding to specific molecular weights to enable probing for different proteins from the same blot. Membranes were probed for APP and Aβ, C-100 and tau. Samples from each age group were normalized against the internal control. Because of the nature of the comparisons to be made, protein levels were measured and age-related effects were constructed by calculating the fold change in protein levels using 3-month-old samples as a baseline reading.