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A novel technique for simultaneous bilateral brain infusions in a mouse model of neurodegenerative disease

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ABSTRACT

A common problem faced by researchers using transgenic models to study disease is the phenotypic variability that exists within a group or colony of animals. Significant pathological analyses thus often require large numbers of mice to perform. Many lines of transgenic mice harboring the gene for human amyloid precursor protein (APP) with different mutations causing familial Alzheimer's disease have been developed over the past decade to study plaque deposition and other aspects of AD. However, variations in size, density, plaque number, and total amyloid load between animals of the same age and genotype have been identified by our lab and others. Therefore, to study the effects of compounds on amyloid pathology, it was imperative to develop a technique that would allow each brain hemisphere to receive different infusions. We have developed catheters that facilitate simultaneous bilateral infusion in mouse brains, thereby using the contralateral hemisphere of the same animal as an internal control while studying, for example, the effect of compounds on amyloid plaques, a pathological hallmark of the progression of Alzheimer's disease (AD). Several molecules have been identified within the plaques including the major component, the Aβ peptide, and two inflammation-related proteins, apolipoprotein E (apoE) and the serine protease inhibitor α-1-antichymotrypsin (ACT). In these experiments, ACT was infused unilaterally over a period of 28 days into the parenchyma and lateral ventricles of PS/APP mice and observed to associate with amyloid plaques, with minimal mortality. Utilizing the ACT/Aβ interaction, details of this procedure are discussed here in detail.

1. Introduction

Alzheimer's disease is the most common form of neurodegeneration, affecting over 5 million people nationwide. Approximately 1 person in 8 will be diagnosed with AD by the age of 65, and 1 in 2 by the age of 85 (National Institutes of Health U.S. Department of Health and Human Services). The disease is characterized by severe cognitive decline, leading to dementia and death within an average of 8 years from diagnosis. Pathologically, the disease outcome is massive neuronal loss, extracellular deposits of amyloid, and intracellular accumulation of neurofibrillary tangles (Selkoe, 1986). Several proteins have been identified within the plaques including Aβ, apolipoprotein E (apoE) (Wisniewski and Frangione, 1992) and α-1-antichymotrypsin (ACT) (Abraham et al., 1988). Aβ is the primary component of the plaques and is a 38–43 amino acid peptide proteolytically derived from the amyloid precursor protein (APP) (Hardy and Selkoe, 2002). Mutations in the APP gene that cause AD result in an increased production of pathogenic forms of Aβ (Tanzi et al., 1991). ApoE and ACT are part of a local inflammatory process in the brain. Aβ binds to ACT and ApoE, and these proteins serve to catalyze the conversion of Aβ into filaments *in vitro* (Ma et al., 1996; Wisniewski et al., 1994), and *in vivo* to form beta-pleated structured amyloid deposits (Bales et al., 1999; Nilsson et al., 2001).

ACT is a 68 kDa acute phase serum glycoprotein from the family of serine protease inhibitors (serpins). Systemically, it is released by hepatocyes and monocytes in response to inflammation and functions as a suicide inhibitor of cathepsin G released by neutrophils (Kalsheker, 1996). In the brain, ACT is known to be produced by and released from astrocytes in response to IL-1β stimulation by microglia following trauma, infection, or amyloidoses (Abraham, 2001; Das and Potter, 1995). ACT is highly upregulated in AD brain and is involved both in the formation of amyloid

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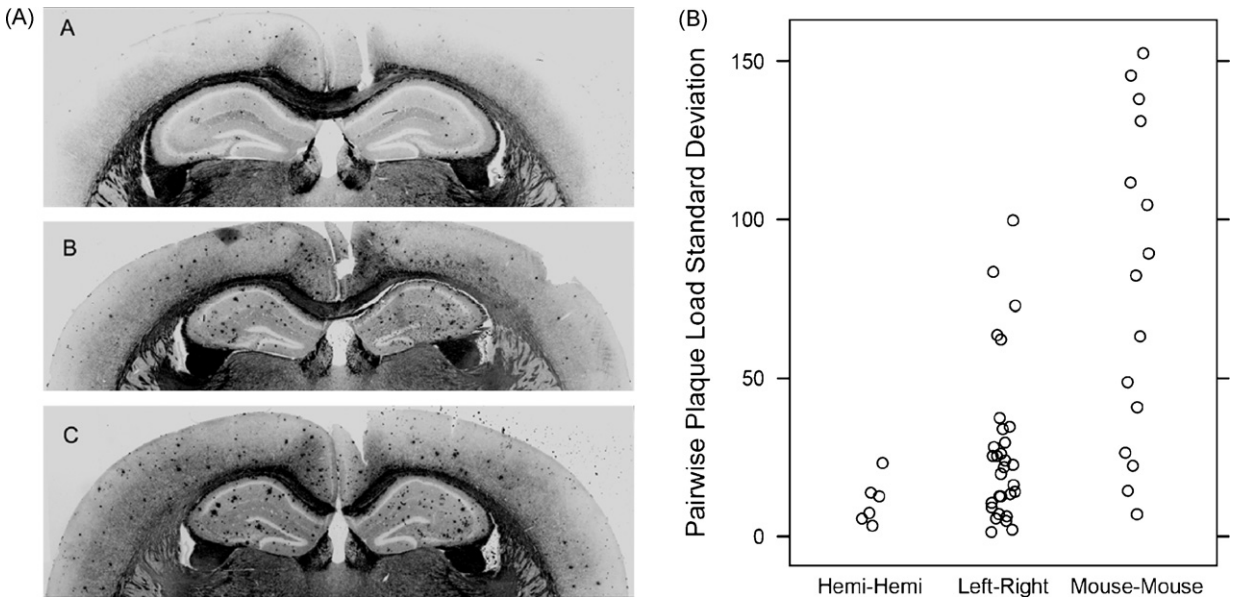

Fig. 1. (A) Thioflavin S staining on 25 μm coronal sections (montages) of 3 different PS/APP mice at 10 months of age showing plaque variability in the hippocampus and neighboring cortex. (B) Scatter-plot of plaque load data from 5 brain sections through 6 ten-month-old PS/APP mice comparing standard deviation (plaque variance) between total hemispheres of the same animal (28%), between sides of the same section (11%) and between animals (78%). All of the data from the left hemisphere are compared with the right for each mouse (Hemi–Hemi, 1 data point for each mouse); the left half of a single slice is compared with the right side (Left–Right, 1 data point for each slice); and a permuted comparison of each mouse with every other mouse (Mouse–Mouse, 1 data point for each pair of mice). Where data were aggregated (Hemi–Hemi and Mouse–Mouse), the mean of the aggregated counts was used to make the standard deviation comparable to the single slice data (standard deviation of two numbers).

plaques (Abraham et al., 1988) and in the phosphorylation of tau (Padmanabhan et al., 2006). Using a transgenic mouse carrying a mutated human presenilin gene (PS) and a mutated human APP gene (PS/APP), we observed the co-localization of ACT on existing amyloid plaques in adult PS/APP mice, utilizing a novel brain infusion technique.

Amyloid plaque load can vary significantly from animal to animal of the same APP transgenic genotype as well as from hemisphere to hemisphere of the same animal (Fig. 1). We analyzed the brains of 6 PS/APP mice at 10 months of age at 5 sections per brain front to back for variations in plaque load. Although variability does exist from hemisphere to hemisphere in this model per a given section (11%), we have demonstrated that the variability from animal to animal is almost 3 times that from that of total hemisphere to hemisphere comparison (78% from mean compared to 28%, respectively) (Fig. 1B). For this reason, we designed a bilateral delivery technique that would allow one of the two hemispheres to serve as an internal control. This technique allows researchers to use fewer mice than an experiment of this nature would typically require and with more accurate results. Thus ACT, for the purposes of our work, could be delivered to one hemisphere and the vehicle, artificial cerebrospinal fluid (aCSF), to the other. Current systems for drug/compound delivery to the mouse brain use pedestal cannulae which are commercially available, but tend to be bulky and irritating to the animal, restrictive to the researcher, and lead to high mortality rates. Furthermore, use of two independent pedestal-based cannulae infusions in the same mouse is not possible due to their large size. Therefore, we designed and constructed novel catheters that are implanted subcutaneously and contoured to the skull. They are minimally invasive and virtually eliminate iatrogenic mortality. Our data show that this method is reliable for studying the chronic delivery of ACT into PS/APP mice to study its effects on amyloid pathology. Moreover, because the catheters are custom made, they can easily be adapted to study the impact of virtually any compound in other mouse models of neurodegeneration or brain disease as well as on non-transgenic mice in order to study the effects of these compounds on normal physiology.

2. Materials and methods

2.1. Catheter construction

The catheters (patent pending PCT/US08/73974) used for this experiment were made by first taking a 1.25 cm length of 30 gauge stainless steel tubing (Small Parts Inc., Miramar, FL) and carefully inserting it into a 20 g needle (tip removed) to the appropriate depth under a dissecting microscope (Leica, Heerbruug, Switzerland), and bending it at 2.5 mm to approximately 90° being very careful not to crimp the tube. The remaining length of the tube was bent again at 5 mm to an angle of 120–160°, approximating the contour of each animal's skull. These metal cannulae were inserted into a 3 cm length of polyethylene (PE-10) tubing with an internal diameter of 0.28 mm and an outer diameter of 0.61 mm. One centimeter of the PE-10 was then itself inserted into a 4.5 cm length of sterile polyvinyl tubing (PV-50-I.D. 0.69 mm/O.D. 1.14 mm, Durect Corp., Cupertino, CA), held in place using a bead of Locktite 454 adhesive (Plastics One, Roanoke, VA), and cured overnight. The following day, sterile water was forced through each catheter assembly to ensure that the lines were not obstructed. The osmotic pumps were then filled with solution, attached to the catheters and primed and implanted as described below. The proven catheters were later custom manufactured by Braintree Scientific (Braintree, MA) with some minor changes: The adhesive was supplanted by heat sealing PE-10 to PE-50 tubing and, because the diameters of the PV-50 and new PE-50 were not exactly the same, a sheath of silicone (SIL 047) was placed over the PE-50 with an extra 2 mm overhang to attach it to the flow modulator of the pump, forming a tight, leak-proof seal (Fig. 2).

2.2. Transgenic mice

PS/APP (presenilin 1/amyloid precursor protein) mice and PS/APP/ACT mice were generated by crossing heterozygous PDGF-hAPP (V717F) mice with PDGF-hPS1 (M146L) on both Swiss Webster and C57BL/6 backgrounds. In some cases, PS/APP mice