Supplement

Inhibition of δPKC protects against hypertensive encephalopathy

Method and Materials

Immunohistochemistry:

Formalin-fixed, paraffin-embedded postmortem human brain tissue sections (5 M thick) were deparaffinised with xylene, dehydrated with graded alcohol, and treated with 3% hydrogen peroxide in methanol for 5 min to block endogenous peroxidase activity. The sections were then subjected to antigen retrieval by irradiating in target retrieval solution (Dako, Carpinteria, California, USA) for 5 min in a microwave oven. Normal goat serum (10%) in PBS was then applied for 20 min to prevent non-specific binding. The sections were incubated with anti- δPKC (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in PBS overnight. After the immunohistochemical reaction, sections were stained with an HRP IHC kit (Chemicon, Temecula, CA, USA). Finally, the sections were dehydrated through graded alcohols and xylene, and coverslipped.

Figure legend:

Supplemental Fig 1: Immunohistochemical analysis of δPKC postmortem samples of human brain. Paraffin-embedded sections of cerebral cortex and basal ganglia from three human brains were sectioned (5 M thick) and immunostained with rabbit antibody to δPKC (diluted 1:100 in PBS). Three male patients were studied. 07A22 (86 years old) had a history of hypertensive hemorrhage and died of hypoxic-ischemic encephalopathy. Patient 07A29 (88 years old) also had hypertensive hemorrhage and died of cerebral arteriosclerosis. The third patient (07A31, 61 years old) had no history of hypertension and died of amyotrophic lateral sclerosis (normal basal ganglia and cortex were observed). Two of the patients (07A22 and 07A29) had histories of hypertension and cerebral infarcts. The control patient (07A31) was

normotensive. Immunostaining for δPKC was observed in smooth muscle of microvessels in all three patients. Increased staining in astrocytes (arrowheads) and endothelial cells (arrows) was observed in the cases with histories of hypertension. Four representative vessel cross-sections from each patient are shown.

Supplementary Figure 1