Protection Against HIV-1 gp120–induced Brain Damage by Neuronal Expression of Human Amyloid Precursor Protein

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Summary

Expression of the HIV-1 envelope protein gp120 in brains of transgenic (tg) mice induces extensive neurodegeneration (Toggas, S. M., E. Masliah, E. M. Rockenstein, G. F. Rall, C. R. Abraham, and L. Mucke. 1994. Nature [Lond.], 367:188–193.). To further analyze the pathogenesis of gp120-induced neurotoxicity and to assess the neuroprotective potential of human amyloid precursor proteins (hAPPs) in vivo, different hAPP isoforms were expressed in neurons of gp120/hAPP-bigenic mice: hAPP751, which contains a Kunitz-type protease inhibitor domain, or hAPP695, which lacks this domain. Bigenic mice overexpressing hAPP751 at moderate levels showed significantly less neuronal loss, synapto-dendritic degeneration, and gliosis than singly tg mice expressing gp120 alone. In contrast, higher levels of hAPP695 expression in bigenic mice failed to prevent gp120-induced brain damage. These data indicate that hAPP can exert important neuroprotective functions in vivo and that the efficiency of this protection may depend on the hAPP isoform expressed and/or on the level of neuronal hAPP expression. Hence, molecules that mimic beneficial APP activities may be useful in the prevention/treatment of HIV-1–associated nervous system damage and, perhaps, also of other types of neural injury.
forms were expressed in neurons of gp120/hAPP-bigenic mice. Our results demonstrate that moderate levels of hAPP751 expression effectively protect the CNS against gp120-induced neuronal injury.

Materials and Methods

Animals and DNA Analysis. Male and female B6xSJL mice (4–14 mo old) were used. Animal care was in accordance with institutional guidelines. Transgenes were detected by slot blot analysis of genomic DNA extracted from tail biopsies using 32P-labeled probes that recognize either gp120 (6) or an SV40 sequence at the 3' end of hAPP-encoding constructs (17).

Expression of Transgene Products. Transgene-derived mRNAs were detected by solution hybridization and RNase protection assay, carried out essentially as described (18), using 10 µg of RNA per sample in combination with the following 32P-labeled antisense riboprobes (protected sequences indicated in parentheses): APP (nucleotides 2468–2657 of APP mRNA [GenBank accession number X06989]), gp120 (nucleotides 2532–2656 of SV40 [GenBank accession number M24914]) at the 3' end of glial fibrillary acidic protein (GFAP)-gp120-derived transcripts, and β-actin (nucleotides 480–559 of mouse β-actin mRNA [GenBank accession number X03672]). hAPP protein expression was detected by Western blot analysis as described (17).

Quantitative Immunohistopathological Analysis. Mice were 4–14 mo of age and there were no significant differences in the average age of mice across the different groups compared by one-factor analysis of variance (ANOVA). Brains were fixed, sectioned, (immuno) stained, and analyzed as described previously (6, 17). Hemibrains were assigned code numbers (by E. M. Rockenstein) to ensure objective assessment. Codes were not broken until the analysis was complete. For each mouse and immunostain, three serial sections of corresponding brain regions were analyzed. For the assessment of neuronal changes, sections were examined using a laser scanning confocal microscope (MRC-600; Bio-Rad Labs., Richmond, CA) (19, 20) mounted on an Axiovert Zeiss microscope (Carl Zeiss, Inc., Thornwood, NY). Digitized images (4 section/case, 0.5 µm in thickness, were transferred to a Macintosh Ile, running the public domain program of Wayne Rasband (Image 1.23) (20). The area of the neuropil occupied by MAP-2-positive dendrites and microglia (Figs. 2 and 3). Whereas brains of gp120 singly tg neonates were indistinguishable at the structural level from brains of non-tg littermate controls, neuronal damage (distortion of apical dendrites and decrease in the area of neuropil occupied by MAP-2–positive dendrites) was evident in 7-d-old gp120 tg mice but not in non-tg littermate controls (data not shown). This early development of brain damage in gp120 tg mice is consistent with the postnatal increase in expression directed by the GFAP promoter (24). Notably, it also correlates well with the developmental expression of NMDA receptors (25), which appear to play an important role in gp120-induced neurotoxicity (2). The early development of neuropathology in gp120 tg mice implies that, to be effective, preventative therapeutic interventions may have to be initiated before or shortly after birth. Previous studies (26) have shown that the NSE promoter is active before birth making it suitable for the expression of potentially neuroprotective factors in gp120 tg mice.

To generate gp120/hAPP695 and gp120/hAPP751 bigenic mice, gp120 singly tg heterozygous mice were crossed with either hAPP695 or hAPP751 singly tg-heterozygous mice. Following Mendelian genetics, the offspring from such crosses were singly tg for either hAPP or gp120, bigenic for gp120/hAPP or non-tg, each group comprising ~25% of any given litter. Brains of bigenic mice were compared quantitatively with brains of non-tg or singly tg littermates. Moderate levels of neuronal expression of hAPP751 in gp120/hAPP bigenic mice significantly decreased the neuronal loss and synapto-dendritic damage found in singly tg mice expressing gp120 alone (Fig. 2, A and B). This protection was so effective that the structural integrity of neurons
in gp120/hAPP751 bigenic mice was essentially indistinguishable from that in non-tg controls (Fig. 3). In contrast, neuronal expression of hAPP695 at higher levels had no neuroprotective effects (Figs. 2 and 3).

Although gp120/hAPP751 bigenic mice also showed significantly less gliosis (astrocytosis and microgliosis) than gp120 singly tg mice (p <0.002), the inhibition of the gliosis was clearly incomplete (Fig. 2 C). It is possible that the gliosis in gp120/hAPP751 bigenic mice represents a residual glial response to chemical distress signals from neurons that appear structurally intact but are functionally impaired, or results from more direct effects of gp120 on astrocytes and/or microglia.

In vitro, gp120 or gp120-induced mediators appear to induce excitotoxicity by synergizing with glutamate to elevate neuronal intracellular-free calcium levels ([Ca^{2+}]_{i}) (27), whereas secretable forms of hAPP diminish the glutamate-induced rise in [Ca^{2+}]_{i} (28). Although there is currently no reliable method to directly measure neuronal [Ca^{2+}]_{i} in vivo, our demonstration of neuroprotective hAPP effects in gp120/hAPP bigenic mice indicates that these molecules may have similar effects in vivo. It is interesting in this context that [Ca^{2+}]_{i}-imaging of cultured neurons revealed the APP-mediated reduction in neuronal [Ca^{2+}]_{i} to be particularly marked in dendrites (28), since these neuronal structures show prominent damage in gp120 tg mice (Figs. 2, A and B and 3). Because in aggregated form the APP derivative Aβ has calciumdestabilizing and neurodegenerative effects in vitro (29), it is important to note that none of our NSE-hAPP tg lines (age range of mice analyzed: 2–24 mo) showed evidence for...
Figure 2. Computer-aided quantitation of neuroprotective hAPP effects. Hemibrains of singly tg (hAPP751 vs. hAPP695 vs. gp120), bigenic (gp120 + hAPP751 vs. gp120 + hAPP695), and non-tg mice (6-11 mice analyzed per group) were fixed, sectioned, and either immunolabeled with antibodies against MAP-2 (neuronal dendrites), synaptophysin (presynaptic terminals), GFAP (astrocytes), or F4/80 (macrophages/microglia) to analyze structural features, or stained with Cresyl violet to determine counts of large pyramidal neurons. Quantitative assessments were carried out as described in Materials and Methods. To facilitate comparisons across different groups of mice and different neuronal/glia parameters, for each parameter, the median of measurements obtained in 6-11 non-tg controls was used as the normal baseline value and arbitrarily defined as 100%. The data shown in A and C represent deviations (mean increase or loss ± SEM) from this non-tg baseline. The extent of vacuolization of neocortical neuronal dendrites (B) was graded semiquantitatively (0, none; 1, mild; 2, moderate; 3, intense) based on the inspection of confocal images from three MAP-2-immunostained sections per case. Only the columns marked by asterisk(s) showed statistically significant differences from results obtained in normal non-tg controls: ** p < 0.01, * p < 0.05.

Aβ/amyloid deposits or neurodegeneration when examined with a variety of antibodies that readily detect such alterations in brains of patients with Alzheimer's disease (17).

Although it is reasonable to postulate that gp120-induced neurotoxicity was prevented in gp120/hAPP bigenic mice by hAPP-mediated stabilization of the intraneuronal calcium homeostasis, alternative mechanisms also deserve consideration. Coexpression of hAPPs in bigenic mice did not significantly alter the cerebral levels of gp120 mRNA compared with gp120 singly tg mice (Fig. 1 B). However, it has so far been difficult to unequivocally identify soluble gp120 in brains of gp120 tg mice or in brains of patients with HIV-1 encephalitis (6) and the current study was not designed to evaluate whether cerebral hAPP expression affects gp120 protein levels or alters the concentration of gp120-induced neurotoxic mediators. Both hAPP695 and hAPP751 contain a metalloprotease inhibitor domain (30) and protease inhibitors could mediate a variety of important biological effects in the CNS (31, 32). Because hAPP751 expression protected against gp120-induced neurotoxicity, whereas hAPP695 expression did not, one might be tempted to speculate that this difference relates to an activity of the KPI domain that is present in hAPP751 and absent from hAPP695. However, the two hAPP lines evaluated in the current study also differed with respect to their level of hAPP expression, line NSE-hAPP695m-19 showing significantly higher levels of hAPP expression than line NSE-hAPP751m-57 (Fig. 2, B and C). Notably, a more extensive investigation of synaptotrophic hAPP effects in multiple lines of hAPP tg mice suggested that the dose-response curve for potentially beneficial hAPP effects might be bell-shaped with progressively less neurotrophism/protection seen at higher levels of expression (17). Experiments are currently in progress (a) to confirm neuroprotective hAPP effects in additional hAPP tg lines, (b) to differentiate KPI domain-related from hAPP dosage effects, and (c) to compare the neuroprotective capacity of wild-type versus mutated hAPPs.

In conclusion, our in vivo results are consistent with the postulate that gp120-induced neurotoxicity involves derangements of the neuronal calcium homeostasis. The prominent
Figure 3. Neuroprotective hAPP effects revealed by confocal microscopy of brain sections (frontal cortex) immunolabeled with antibodies against the neuronal dendritic marker MAP-2 (green) or the astroglial marker GFAP (red). Hemibrains of non-tg, singly tg, and bigenic mice were paraformaldehyde fixed, sectioned, immunostained, and analyzed by laser scanning confocal microscopy as described (6). Compare the normal appearance of neurons and paucity of astroglial activation in non-tg controls and gp120/hAPP751 bigenic mice with the rarefaction and vacuolization (arrows) of neuronal dendrites and the reactive astrocytosis seen in gp120 singly tg and gp120/hAPP695 bigenic mice.

The neuroprotective effect of hAPP demonstrated here indicates that drugs that mimic beneficial APP activities might be useful in the treatment or prevention of HIV-1-associated nervous system damage and, perhaps, also of other types of neural injury.

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