Amyloid precursor protein in the cerebral cortex is rapidly and persistently induced by loss of subcortical innervation.
Amyloid precursor protein in the cerebral cortex is rapidly and persistently induced by loss of subcortical innervation
(nucleus basalis of Meynert/rat)

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ABSTRACT Lesions of the cholinergic nucleus basalis of Meynert elevate the ex vivo synthesis of β amyloid precursor protein (β-APP) in the cerebral cortex, a major projection region. We have found that this elevation is reflected by increased levels of β-APP mRNA. The induction is rapid (occurring 60 min after placement of the lesion) and persistent (remaining for at least 45 days after lesioning). Two other subcortical lesions, which result in reductions of cortical adrenergic and serotonergic innervation, similarly induced cortical β-APP. The β-APP induction is reversible and does not require loss of the subcortical neurons. Infusion of lidocaine, a calcium antagonist that disrupts neurotransmitter release, into the nucleus basalis of Meynert leads to the temporary reduction of released acetylcholine in the cortex. In this model, β-APP mRNA levels are elevated shortly after the infusion of lidocaine (90 min) but return to preinfusion levels 7 days after the lidocaine treatment. However, metabolic stresses of the brain, including chronic physostigmine, glucocorticoid, and diabeticogenic treatments, fail to induce the β-APP response. These results suggest that the induction of β-APP is a specific response to the loss of functional innervation in the cortex. Importantly, these studies show that cortical β-APP is induced by lesions that mimic the neurochemical deficits most frequently observed in Alzheimer disease.

Among the most prominent features of Alzheimer disease (AD) are profound deficits in cortical cholinergic, noradrenergic, and serotonergic neurotransmitters and the association of amyloid peptide with senile plaques. The reductions of the cortical neurochemical markers for these neurotransmitters appear to be due to the loss of hypofunction of the subcortical neurons responsible for the cortical innervation (1). Amyloid peptide derives from a larger β amyloid precursor protein (β-APP) by an as yet uncharacterized mechanism (2). This observation has led to the proposal that production of the amyloid peptide may be due to selective overexpression of β-APP, particularly in those cortical areas that exhibit an abundance of senile plaques. The relationship between neurotransmitter deficits and β-APP expression has not been elucidated and a unitary paradigm for studying these apparently diverse neuropathological features has not emerged.

We have begun to investigate the relationship between β-APP gene expression and neurotransmitter deficits by determining the effect of neurotransmitter depletion on ex vivo β-APP synthesis and β-APP mRNA levels in the cerebral cortex of the rat. Previously, we have shown that lesions of the nucleus basalis of Meynert (nbM) result in increased ex vivo synthesis of cortical β-APP 7 days after placement of the lesion (3). The elevated synthesis exhibited specificity to β-APP in that numerous other proteins, including glial fibrillary acidic protein, were not affected.

We have now characterized this lesion-induced β-APP expression more completely and report that (i) the elevated ex vivo synthesis is due to increased levels of β-APP mRNA; (ii) the β-APP response to the lesion is rapid (exhibited within 60 min of lesioning) and persistent (remaining as long as 45 days post-lesion); (iii) the induction is reversible, requiring the attenuative decrease in neurotransmitter release but not the loss of the subcortical neurons; (iv) subcortical lesions of the cortically projecting noradrenergic and serotonergic systems similarly induce cortical β-APP expression; and, finally, (v) other general perturbations of central nervous system function fail to induce the elevated β-APP synthesis response. These results suggest a cause and effect relationship between lesion-induced neurotransmitter deficits and β-APP induction, providing a possible linkage between subcortical neurotransmitter system deficits and amyloid deposition in the AD brain.

METHODS

Placement of Lesions. Adult (∼8 weeks old) male Sprague-Dawley rats (=225–250 g) purchased from Charles River Breeding Laboratories were subcortically lesioned at the following sites: (i) unilateral lesions of the nbM with N-methyl-d-aspartate (NMDA, 50 nM) as the excitotoxin as described (3); (ii) unilateral lesions of the ascending noradrenergic bundle (ANB) with 6-hydroxydopamine (2 μl of a 4 mM solution) as described (4); (iii) dorsal raphe nucleus (DRN) lesions with 5,7-dihydroxytryptamine (50 mM) as described (5). Controls for the nbM and ANB lesions were the contralateral cortices, whereas controls for the DRN lesions were sham-operated animals. This latter sham-operated group also served as controls for any nonspecific contralateral effects produced by the unilateral nbM and ANB lesions.

Transient Inhibition of Cortical Acetylcholine (ACh) Release. A total of 12 male Long Evans hooded rats (300–325 g) was used in this experiment. Each rat was anesthetized with chloral hydrate (400 mg/kg, i.p.) and was positioned in a Kopf stereotaxic apparatus. A 28-gauge cannula was lowered into the region of the nbM (Bregma ~0.5, ±3.0, ~7.7). One-half of the rats were randomly assigned to group I and also received a 2-mm microdialysis probe (CMA/12; Bioanalytical Systems, West Lafayette, IN) that was stereotaxically directed at the frontal cortex at coordinates Bregma...
RESULTS

Effects of nbM (Cholinergic) Lesions on β-APP mRNA. One week following the lesion of the nbM, mRNA for β-APP was determined by Northern analysis using a cDNA probe that recognizes all β-APP mRNA isotypes (6). The amount of β-APP mRNA was significantly elevated in the RNA samples made from lesioned cortex compared to its control cortex (Fig. 1). The single message present in the samples reflects the predominance of the mRNA encoding the 695-residue form of β-APP (β-APP695) in rat brain (6). No significant changes were observed when similar samples were probed with actin cDNA. Statistical analysis of the results for the ratio of the densitometric values for β-APP/actin revealed highly significant differences of a transient lesion and control cortices [t(5) = 4.7, P < 0.005]. The mean and SEMs for control and lesioned cortices were 0.48 ± 0.06 and 1.05 ± 0.13 densitometric units, respectively. This result indicates an overall elevation of β-APP mRNA concomitant with the elevated ex vivo synthesis of the protein (see below and ref. 3).

Time Course of Cortical β-APP Induction by nbM Lesions. Rats receiving unilateral nbM lesions were sacrificed at various times after lesioning. The level of β-APP synthesis was then measured as synthesized by polyribosomes. Ex vivo synthesis of β-APP was significantly increased in the lesioned cortex at all time points after 5 min and up to 45 days after neurotoxin infusion (Fig. 2). A lesion by survival time analysis of variance revealed a significant main effect of lesion [F(1/27) = 83.7, P < 0.0001] and a significant lesion by survival time interaction term [F(5/27) = 4.85, P = 0.003]. Aliquots of the tissue homogenates were assayed for the cholinergic marker enzymes Ch acetyleholinesterase (ChAT) by the method of Fonnum (8). The results of this assay (Fig. 3) demonstrate that ChAT activity decreased steadily until day 3 post-lesion, at which time asymptotic levels were reached. Analysis of variance and Newman–Keuls post-hoc tests revealed a significant effect of survival time [F(5/30) = 39.4, P < 0.0001]. The infusion of NMDA into the nbM led to a significant decrease in cortical ChAT activity at 1 day post-lesion (P < 0.01) followed by a further decrement (P < 0.01) in ChAT activity in the third day post-lesion. Cortical ChAT activity was not affected by lesions placed 5 min or 1 hr prior to sacrifice (P > 0.4). The lesion-induced ChAT deficits persisted unchanged for the 6-week post-lesion period studied. These results demonstrate that the induction of β-APP synthesis by nbM lesions is time dependent and persists for at least 6 weeks post-lesion (Figs. 2 and 3).

Transient Induction of β-APP by Transient Inhibition of ACh Release. The basal release of ACh in the frontal cortex following the infusion of lidocaine into the nbM was compared to ACh release during the 20-min baseline period immediately preceding lidocaine infusion (Fig. 4A). The initial infusion of lidocaine resulted in an average decrease of

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\text{C L C L C L}
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\[
\beta-APP
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\[
\text{Actin}
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\text{\textbf{FIG. 1.} Levels of β-APP and actin mRNA in control (C) and lesioned (L) cortices. Rat nbM were unilaterally lesioned and total RNA was isolated from contralateral (control) and ipsilateral (lesioned) cortex 7 days later, as described in the text. Total RNA (10 μg) was separated on 1% agarose/formaldehyde gels, blotted onto nitrocellulose filters, and probed with cDNA to either β-APP (Upper) or actin (Lower). The filter was then exposed to film for 4 days. Shown are three representative control/lesion pairs (of six that were examined).}
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ACh release by 25%. Mean ACh release then recovered to near baseline levels during the 60-min period following the first infusion of lidocaine. The time course of maximal ACh release inhibition was variable across different animals. Mean maximal ACh release inhibition was 39.8% of baseline. An analysis of variance comparing the two groups of animals across the post-lidocaine infusion 10-min time epochs revealed a significant effect of lidocaine treatment condition ($F(1/50) = 13.9, P < 0.0008$). Post-hoc comparisons (Newman-Keuls) revealed that frontal cortical ACh release was significantly ($P < 0.05$) inhibited during the first three 10-min recording epochs following lidocaine infusion, relative to the same time points in the animals in which ACh release was measured 1 week following lidocaine infusion. Levels of $\beta$-APP mRNA in the cortex were assayed in animals sacrificed either 90 min or 7 days after lidocaine infusion. Total RNA was isolated from cortices ipsilateral (L) and contralateral (C) to the nbM infused with lidocaine. $\beta$-APP mRNA was determined on Northern blots. Shown are three representative pairs (of six assayed) each of the short-term-survival and long-term-survival samples. The three short-term RNA samples assayed for actin mRNA are shown (B).
The adrenalectomized animals were treated chronically either with saline or with a high dose of the potent type II adrenal-steroid receptor agonist RU 28362 (9) (administered by Alzet mini pumps at 10 μg/hr for 7 days; n = 5). (iii) In collaboration with S. Hoyer (Heidelberg University, Germany), different groups of rats were treated intracerebroventricularily either with artificial cerebrospinal fluid (aCSF) or with aCSF plus the diabetogenic compound streptozotocin (1.25 μg/g of body weight; n = 4) for 42 days, resulting in significant reduction of brain energy metabolism as indicated by a 47% decrease in ATP/ADP ratios (10). The rats in all three studies were sacrificed, cerebral cortices (for the physostigmine and streptozotocin experiments) or cerebelli (for the adrenalectomy and RU 28362 experiment) were dissected, and β-APP synthesis was investigated by the polyclone translation methods described in Materials and Methods. Analysis of the results (Table 2) revealed that none of the treatments significantly affected the ex vivo synthesis of β-APP relative to untreated controls (all r < 1.1, all P > 0.1).

### DISCUSSION

In an initial investigation, we found that lesions of the nbM resulted in increased synthesis of β-APP by purified polymersomes in the cortical projection areas 7 days after lesioning (3). Polysomes that are isolated from tissue contain mRNA in the process of synthesizing their corresponding polypeptides in vivo. Therefore, the increased synthesis of β-APP by the lesioned cortical polysomes suggests that β-APP synthesis is elevated in vivo. However, because the cortical polysomal mRNA is translated in vivo in a heterologous assay system, it is possible that β-APP mRNA is in some way preferentially translated and does not accurately reflect the rate of β-APP synthesis in vivo. This induction of cortical β-APP has been further characterized to better understand the normal role of β-APP in the intact brain and the potential role of β-APP in subsequent pathological processes.

The increased synthesis of β-APP by cortical polysomes in the lesioned animal was corroborated by the observation of elevated β-APP mRNA in the lesioned samples (Fig. 1). The increased mRNA suggests that the expression of β-APP was due to the greater transcription of the β-APP gene, although our observations could also be explained by enhanced stability of the β-APP message after the lesion. Due to its predominance in the rat brain (6), only the β-APP_{225} splice variant was directly examined in this assay. The equal elevation of each protein isotype as seen in the immunoprecipitates suggests that all splice variants are induced with the lesion.

The time course of the β-APP induction shows that it is rapid and persistent (Fig. 2). The initial induction of β-APP at 1 hr may be obtained by either transcriptional or translational up-regulation, or both. The rapid induction of β-APP post-lesion suggests that the induction of β-APP is not a function of the physical loss of the cortical cholinergic synapse. Since cortical ChAT activity, and presumably the

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### Table 1. Effects of lesions of nbM, ANB, and DRN on cortical neurotransmitter markers and β-APP synthesis

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Transmitter marker*</th>
<th>β-APP synthesis†</th>
<th>%†</th>
</tr>
</thead>
<tbody>
<tr>
<td>nbM control</td>
<td>39.1 ± 1.3</td>
<td>3.05 ± 0.42</td>
<td>225</td>
</tr>
<tr>
<td>nbM lesion</td>
<td>18.1 ± 0.98</td>
<td>6.84 ± 0.53</td>
<td>54</td>
</tr>
<tr>
<td>ANB control</td>
<td>0.25 ± 0.01</td>
<td>3.57 ± 0.39</td>
<td>34</td>
</tr>
<tr>
<td>ANB lesion</td>
<td>0.13 ± 0.01</td>
<td>6.30 ± 0.52</td>
<td>180</td>
</tr>
<tr>
<td>DRN control</td>
<td>0.12 ± 0.005</td>
<td>2.67 ± 0.25</td>
<td>34</td>
</tr>
<tr>
<td>DRN lesion</td>
<td>0.05 ± 0.005</td>
<td>5.74 ± 0.62</td>
<td>224</td>
</tr>
</tbody>
</table>

*For nbM, ChAT (nmol of ACh per hr per mg of protein); for ANB, norepinephrine (μg/g of wet weight); for DRN, serotonin (μg/g of wet weight).

†% of control.

§ Densitometric units per total 35S-labeled protein.

### Table 2. Effects of various stresses on β-APP synthesis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>β-APP synthesis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physostigmine</td>
<td>5</td>
<td>4.27 ± 0.27</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>4.67 ± 0.55</td>
</tr>
<tr>
<td>Glucocorticoid</td>
<td>4</td>
<td>1.88 ± 0.64</td>
</tr>
<tr>
<td>Sham</td>
<td>4</td>
<td>1.45 ± 0.31</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>1.42 ± 0.25</td>
</tr>
<tr>
<td>Stressotocin</td>
<td>5</td>
<td>2.58 ± 0.66</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>3.24 ± 0.91</td>
</tr>
</tbody>
</table>

*Densitometric units per total 35S-labeled protein.
cholinergic neurons containing it, are lost much more slowly following nBM lesions (11, 12) (Fig. 3: no change in ChAT activity at 60 min post-lesion, vs. maximal β-APP induction at this time point), it is unlikely that the rapid induction of β-APP synthesis by cortical polysomes is due to the physical loss of cortical synapses with the nBM neurons. The immediate and short-term effects of NMDA infusion into the nBM on cortical ACh levels are unclear. However, electrophysiological data suggest that the loss of neurons to 30 doses of NMDA results in an almost immediate depolarization of the affected neurons (13). This observation suggests that the release of ACh at the cortical synapse is prevented very soon after the infusion of NMDA. Therefore, the projection areas in the cortex may be responding specifically to the absence of neurotransmitter at postsynaptic cortical sites with the induction of β-APP. To determine whether physical damage to the subcortical neurons was a necessary condition for the induction of β-APP, the nBM projecting to one cortical hemisphere was infused with lidocaine, a calcium antagonist that reversibly disrupts the cortical release of ACh (14). Immediately after lidocaine infusion, cortical release of ACh was reduced (Fig. 4A). Concomitant with this decrease in cortical cholinergic neurotransmission, there was an elevation of cortical β-APP mRNA levels (Fig. 4B). With time after the infusion of lidocaine, cortical ACh levels returned to normal as did the amount of cortical β-APP mRNAs. These results indicate that (i) a diminution in the release of transmitter in the cortex is sufficient to induce cortical β-APP and (ii) this induction is reversible with the resumption of subcortical neuronal function. These results support the view that the induction of cortical β-APP is triggered by the loss or diminution of neurotransmitter at the postsynaptic site. The reversibility of the induced β-APP in this paradigm suggests that normalization of cortical neurotransmitter activity can reverse the induction of β-APP.

In addition to the forebrain cholinergic deficits in AD, serotonergic and noradrenergic systems are also affected (15). Results from a variety of experiments in animals, such as those reported here, suggest that lesions of the ANB and the DRN produce severe decrements in cortical noradrenergic and serotoninergic markers (4, 5). Such lesions also produce impairments in cognitive function, arousal state, and responsiveness to pharmacological agents (4, 5). Lesions of these structures resulted in a similar induction of β-APP as seen with nBM lesions (Fig. 5). These results indicate that cortical β-APP is induced not only by forebrain cholinergic lesions (3) but also by lesions that affect cortical noradrenergic and serotonergic systems. It is important to note, however, that lesion-induced increases in polysomal β-APP synthesis are not likely to be a direct result of neurotoxins or nonspecific damage to the central nervous system. This conclusion is supported by the fact that polysomal synthesis of β-APP is (i) induced by lesions produced by different neurotoxins with different mechanisms of action (i.e., cholinergic system, NMDA; noradrenergic system, 6-hydroxydopamine; serotonergic system, 5,7-dihydroxytryptamine); (ii) induced by the actual disruption of neurotransmission since sham lesions, including cannula insertion into the lesion site, fail to affect cortical ex vivo β-APP synthesis; (iii) reversible and dependent upon the attenuation of neurotransmitter release; and (iv) not induced in at least one region of the brain, the cerebellum (data not shown), which does not receive a direct cholinergic projection from the nBM. These findings substantiate the relationship between subcortical lesion-induced neurotransmitter deficits and synthesis of β-APP by cortical polysomes. These studies have not established that increased β-APP synthesis is necessarily specific to lesions of only those systems known to be affected in AD. Induction of β-APP may be a physiologic consequence of the general loss of synaptic activity. Lesions of other cortically projecting systems (such as the dopaminergic system) are also likely to induce cortical β-APP.

Despite the induction of cortical β-APP by a variety of lesions, β-APP induction is not a simple response to general stresses. Three different perturbations of cerebral function did not induce β-APP (Table 2). The results of these three negative experiments demonstrate that ex vivo β-APP synthesis is not influenced by the increased availability of ACh in the cortex (physostigmine treatment) or by generalized central or peripheral stressors (RU 28362 and streptozotocin). These experiments cannot exclude the influence of these agents and manipulations on β-APP synthesis in other tissues or completely rule out the influence of other stressors on cortical β-APP. These results do suggest, however, that the induction of β-APP is not a central response to generalized stress or physiological disturbance and is not observed, at least within the parameters thus far investigated, under conditions where specific central nervous system pathways have not been directly disrupted.

These results indicate that cortical projection areas respond to the loss of subcortical innervation with a rapid and persistent induction of β-APP. This observation suggests that one function of β-APP is to regulate interneuronal communication in the intact brain. The determination of the precise physiological signal for the induction will be important for our understanding of the central nervous system response to injury. That the induction of β-APP is dependent upon the diminution of neurotransmitter activity at the cortical synapse suggests that changes in neurotransmitter activity are among the processes that regulate β-APP and raises the possibility that pharmacological agents that enhance cortical neurotransmitter activity may prevent or diminish the induction of β-APP.