INTRODUCTION

Alzheimer’s disease (AD) is an incurable degenerative dementia, which affects several million patients worldwide (1). A small percentage of cases are familial, in that they have been traced to autosomal dominant inheritance of mutations in three genes: the β-amyloid precursor protein (APP) (2), and the presenilins (PS1 and PS2) (3). All these defined causes of AD lead to an increased production of β-amyloid (βAP), a protein that deposits in the senile plaques of the AD brain (4). The triad of senile plaques, neurofibrillary tangles and neuronal death characterizes both sporadic and familial forms of AD (4).

To date, the general view is that βAP plays a causative role in AD and that neuronal loss and dementia follow as a result of βAP toxicity. βAP has been shown to kill neurons in vitro by activating apoptosis (5), and apoptotic neurons have been found both in transgenic mice expressing βAP (6) and in the AD brain (7). Finally, it has recently been demonstrated that βAP vaccination is “therapeutic” in animal models of AD (8). Therefore, understanding the mechanism(s) responsible for βAP-induced apoptosis may be important in developing possible new treatments for AD.

KEY WORDS: Alzheimer’s disease, β-amyloid protein, cell cycle, DNA replication, mitotic proteins, neuronal apoptosis.
What follows is an overview of recent evidence suggesting a link between the process of apoptotic degeneration and the aberrant re-expression of cell cycle proteins in adult neurons of the AD brain (9-15). In particular, we discuss this unusual phenomenon in relation to the mechanism of \( \beta \)AP toxicity.

**CYCLING NEURONS IN AD**

Classically, the re-entry of a quiescent cell into the cell cycle depends on the sequential activation of cyclin (Cyc)/cyclin dependent kinase (CDK) complexes by extracellular proliferative signals (16). The Cyc D/CDK4-6 complexes drive the transition of cells from G0 to G1 phase. The G1/S transition and the S phase progression are controlled by activation of the Cyc E/CDK2 complex and of the Cyc A/CDK2 complex, respectively. The Cyc B/CDK1 complex guides the G2/M transition.

Cyc/CDK complexes that drive the progression of the cycle are regulated in turn by association with CDK inhibitors (CDKI), which function by arresting cell proliferation at specific checkpoints when anomalous (16). Two CDKI families have been identified: the Kip/Cip family members (p21, p27 and p57) regulate the activity of CDK2, CDK4 and CDK6, while the members of the INK family (p15, p16, p18 and p19) inhibit the activity of CDK4 and CDK6 specifically.

Contrary to previous thinking, some cell cycle regulatory elements (i.e., CDKs) have a constitutive activity in neurons. Thus, although postmitotic and highly differentiated cells, neurons could lack only few components essential to the completion of the cell cycle.

Cell cycle proteins, including CDK4 and CDK1, cyclins D, E and B, the nuclear antigen Ki-67, and the CDKIs p16 and p21, are found in neurons from the AD brain (9-15), where they are not necessarily associated with end stages of neurodegeneration. This observation has suggested that mitotic proteins are not just by-products of apoptosis, but that they may somehow intervene in the degeneration of post-mitotic neurons.

A fascinating hypothesis by Nagy et al. (15) attempts to reconcile the presence of cell cycle molecules with AD neuropathology. Based on the observation that AD neurons express either cyclin E or cyclin B, but never cyclin A, the authors propose two alternative possibilities. Neurons that do not cross the G1/S transition might re-differentiate; neurons that enter G2 phase, without replicating their DNA, would either undergo apoptosis, or develop neurofibrillary tangles as a result of a G2 phase-related CDK activity.

Although it has recently been demonstrated that DNA replication occurs in AD neurons (17), the early hypothesis of a death phenotype, which depends on the fate of neurons entering the cell cycle, is still plausible.

**\( \beta \)AP NEUROTOXICITY AND CELL CYCLE**

Given that AD neurons seem to enter a cell cycle before undergoing apoptosis, it is particularly important to determine whether it is \( \beta \)AP that reactivates the cell cycle in neurons. To answer this question we carried out cell cycle studies on post-mitotic rat cortical neurons exposed to \( \beta \)AP. We demonstrated that synthetic \( \beta \)AP fragments [i.e., the full-length \( \beta \)AP (1-42) and the active fragment \( \beta \)AP (25-35)] promote the activation of a cell cycle in differentiated cultured cortical neurons (18) (Fig. 1). \( \beta \)AP activates the cycle in neurons by inducing the sequential expression of cell cycle proteins usually functioning in proliferating cells. In particular, following \( \beta \)AP treatment, we observed the induction of cyclin D1, phospho-retinoblastoma (the substrate of Cyc D/CDK4-6 complexes), cyclin E and cyclin A. More importantly, \( \beta \)AP-treated neurons enter the S phase (i.e., they start DNA replication).
before undergoing apoptosis. Interestingly, cytostatic drugs that act as CDK inhibitors (e.g., mimosine and flavopiridol) are protective against βAP-induced neurotoxicity (Fig. 2). More specifically, we have found that blockade of the G1/S transition using a cyclin D1 antisense or a dominant-negative mutant of CDK2 prevents βAP-induced apoptosis (18).

Giovanni et al. have shown that the development of βAP-induced apoptosis requires the activation of Cyc D/CDK4-6 complexes, whereas a dominant-negative mutant of CDK2 was inactive in their study (19).

In addition, it appears that βAP can also re-activate the cell cycle in neurons through a primary action on microglia. Accordingly, cortical neurons that have been cultured with βAP-activated microglia express cell cycle protein and synthesize DNA (20).

Overall, we can conclude that the re-activation of the cell cycle is an obligatory step in the apoptotic pathway evoked by βAP. Yet it is not clear how neurons exposed to βAP exit the cycle and enter the execution phase of apoptosis.

At present, we can presume that events related to neuronal DNA replication are necessary for the occurrence of βAP-induced apoptosis. Bearing in mind that apoptosis functions to halt proliferation of dividing cells carrying non-repairable DNA modifications, we hypothesize that de novo DNA synthesis occurring in neurons after an unscheduled cell-cycle entry may be per se the source of DNA damage.

The evidence that the DNA damage sensor p53 is expressed in degenerating neurons of the AD brain (21-22) supports this hypothesis. Nevertheless, the link between βAP-induced cell cycle re-activation, DNA damage and p53-dependent apoptosis remains to be demonstrated.

CONCLUDING REMARKS

The emerging concept in this field of research is that cell cycle molecules are an essential component of apoptotic degeneration in AD. In addition to βAP, other factors, such as the loss of synaptic connections between neurons, or excitotoxic phenomena, may contribute to the re-entry of AD neurons into the cell cycle (Fig. 3 see over). Accordingly, cell cycle-related events have been described both in neuronal apoptosis by trophic deprivation (23) and in excitotoxic death (24). From this rapidly
growing field of research, the idea is emerging that the neuronal death pathway proceeds through the reactivation of the cell cycle and, therefore, cell cycle proteins are candidate targets of an unknown class of drugs for AD.

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**REFERENCES**

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