The Choline-Leakage Hypothesis for the Loss of Acetylcholine in Alzheimer's Disease

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ABSTRACT We present a hypothesis for the loss of acetylcholine in Alzheimer's disease that is based on two recent experimental results: that β-amyloid causes leakage of choline across cell membranes and that decreased production of acetylcholine increases the production of β-amyloid. According to the hypothesis, an increase in β-amyloid concentration caused by proteolysis of the amyloid precursor protein results in an increase in the leakage of choline out of cells. This leads to a reduction in intracellular choline concentration and hence a reduction in acetylcholine production. The reduction in acetylcholine production, in turn, causes an increase in the concentration of β-amyloid. The resultant positive feedback between decreased acetylcholine and increased β-amyloid accelerates the loss of acetylcholine. We compare the predictions of the choline-leakage hypothesis with a number of experimental observations. We also approximate it with a pair of ordinary differential equations. The solutions of these equations indicate that the loss of acetylcholine is very sensitive to the initial rate of β-amyloid production.

INTRODUCTION

The brains of patients with Alzheimer's disease (AD) are characterized by the presence of plaques and tangles, by a loss of neurons, and by a deficit of certain neurotransmitters. There is evidence linking each of these characteristics, as well as other effects of AD, to possible disease mechanisms. For example, it has been proposed that amyloid plaques cause AD by increasing intraneuronal calcium concentrations (Hardy and Higgins, 1992); that tangles cause AD by interfering with neuronal plasticity (Callahan and Coleman, 1995); that channels formed from β-amyloid cause AD by providing a leakage pathway for calcium to enter neurons (Arispe et al., 1993); and that acetylcholine (ACh) loss causes AD by leading to the loss of phosphatidylcholine, and thus of the integrity of plasma membranes (Wurtman, 1992).

One of these effects (or several other effects that have been proposed) could be the primary cause of AD. Alternatively, there may be multiple causes. In either event, it is useful to understand the relevant mechanisms. In this paper we propose a specific mechanism for the observed deficit in the concentration of ACh.

In our model, β-amyloid-dependent leakage of choline and ACh-dependent production of β-amyloid are reciprocal effects that result in positive feedback and a large decrease in the concentration of ACh. Several qualitative predictions of this model agree with experiment. To obtain more quantitative predictions, we approximate the model by a pair of differential equations, and solve these equations numerically. The solutions describe the time course of the decline in ACh concentration and its dependence on the initial rate of β-amyloid production.

CHOLINE-LEAKAGE HYPOTHESIS

We propose that an increase in the concentration of β-amyloid in brain tissue causes an increased leakage of choline out of cholinergic neurons, thus decreasing the intracellular choline concentration. Because intracellular choline is rate-limiting for the production of ACh (Tucek, 1985), the leakage of choline would also lead to a decrease in the concentration of ACh. The putative outward leakage of choline corresponds to passive movement of choline down its electrochemical gradient. The intracellular choline concentration of cholinergic neurons is normally orders of magnitude higher than the extracellular choline concentration because of active transport generated by the high-affinity choline uptake system (Koliatsos and Price, 1991). Thus there is a larger contribution to the electrochemical gradient by the chemical gradient tending to drive choline outward than by the electrical gradient tending to drive choline inward.

This proposal was stimulated by evidence that β-amyloid can cause ionic leakage in PC12 cells (Galdzicki et al., 1994) and by the observation that AD patients have reduced concentrations of intracellular choline (Nitsch et al., 1992), despite an increase in the rate at which choline is pumped into their cholinergic cells (Slotkin et al., 1990, 1994).

A decrease in ACh concentration has been shown to have two effects relevant to the concentration of β-amyloid. It causes an increase in the synthesis of amyloid precursor protein (APP) in cerebral cortex, as reflected by increased levels of APP mRNA (Wallace et al., 1993), and it favors

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the processing of APP by means of the β-amyloid pathway (Buxbaum et al., 1994; Hung et al., 1993). Both effects tend to increase the concentration of β-amyloid in response to a decrease in ACh. Thus the proposed decrease in ACh caused by an increase in β-amyloid and the resultant choline leakage would lead to a further increase in β-amyloid and hence a further decrease in ACh, resulting in a positive feedback loop. Because of this positive feedback, eventually there would be a significant decrease in ACh.

COMPARISON WITH EXPERIMENTAL DATA

The large decrease in ACh predicted by the choline-leakage hypothesis is consistent with the dramatic decrease in ACh that has been observed in the cerebrospinal fluid (CSF) of patients with AD relative to age-matched controls (Tohgi et al., 1994). This fractional loss is significantly larger than the fractional loss of choline acetyltransferase activity (Slotkin et al., 1994), indicating that there is not only a loss of cholinergic cells in AD, but also that each surviving cell produces less ACh.

Another prediction of the choline-leakage hypothesis is that the loss of ACh in the brains of AD patients would be accompanied by leakage of choline across their cell membranes. This prediction is consistent with the decreased concentration of intracellular choline in AD patients, evidenced by the decrease in choline observed in the frontal cortex (Nitsch et al., 1992), and the increased concentration of extracellular choline in AD patients, evidenced by the increase in choline observed in the CSF (Elble et al., 1989). Increased choline leakage in the brain cells of AD patients is also consistent with the increased choline leakage observed in their red cells (Butterfield et al., 1985).

It is paradoxical that in AD brains, the intracellular choline concentration is decreased (Nitsch et al., 1992), even though the choline transport rate is increased (Slotkin et al., 1990, 1994). What happens to the extra choline that is pumped into the cholinergic neurons of AD patients? The choline-leakage hypothesis suggests a simple resolution of the paradox: choline leaks out into the extracellular space. According to this view, the increased choline transport is the homeostatic response of cholinergic neurons to a loss of intracellular choline.

MATHEMATICAL APPROXIMATION

The positive feedback inherent in the choline-leakage hypothesis implies that the rate of decrease in ACh is very sensitive to the initial rate of production of β-amyloid. To estimate this sensitivity quantitatively, we attempted to describe the relevant processes mathematically. Although an exact description of these processes is quite complex, if a few simplifying assumptions are made, as described below, the essential aspects can be described by two ordinary differential equations with two variables:

Let $a \equiv$ extracellular concentration of ACh

Let $b \equiv$ extracellular concentration of β-amyloid

We assume that the intracellular concentrations of choline and acetylcholine are proportional to each other (Tucek, 1985) and to the extracellular concentration of acetylcholine. We also assume that the membrane concentration of β-amyloid is proportional to the extracellular concentration of β-amyloid. Then

$$da/dt = -k_1 ab \quad (1)$$

$$db/dt = k_2 - k_3 a - k_4 b \quad (2)$$

where k_1, k_2, k_3, and k_4 are constants.

Equation 1 describes the loss of intracellular choline. Because of the simplifying assumptions, this rate is proportional to the time derivative of a. We assume that this rate is also proportional to the intracellular concentration of choline and to the membrane concentration of β-amyloid, as has been shown for PC12 cells (Galdzicki et al., 1994). Because of the simplifying assumptions, these concentrations are proportional to a and to b, respectively. Equation 2 describes the increase in the extracellular concentration of β-amyloid resulting from its formation from APP (represented by the term k_2), the effect of extracellular ACh on the rate of formation (Buxbaum et al., 1994; Hung et al., 1993; Wallace et al., 1993) (represented by the term $-k_3 a$), and the decrease in the extracellular concentration of β-amyloid resulting from enzymatic breakdown and absorption into neuronal membranes (represented by the term $-k_4 b$). The simultaneous solution of these two equations provides a description of the time course of the decline in ACh concentration and the increase in β-amyloid concentration.

The simplified model described above considers the reduction in ACh concentration caused by choline leakage, but does not consider any additional reduction that might be caused by the subsequent death of cholinergic neurons (Yankner et al., 1990).

We used the Mathematica program to obtain numerical solutions for the variables a and b. The assumed initial value for b is zero. The initial value of a and the values for k_1, k_3, and k_4 were chosen to give concentrations of β-amyloid and ages for loss of acetylcholine that are close to physiological values; these are indicated in the legend of Fig. 1. A range of values was chosen for k_2, the rate of β-amyloid production that would occur if no ACh or β-amyloid were present, because the numerical solutions were found to be very sensitive to the value of k_2.

Fig. 1 shows the time course of the decline in ACh concentration according to the simplified model. It can be seen that ACh declines relatively slowly at first, and more rapidly at a later age. Measurement of the CSF ACh concentration in AD patients and in age-matched controls shows that the decline in CSF ACh concentration approximately parallels the severity of dementia (Davis et al., 1982; Tohgi et al., 1994) and that a reasonable estimate of the ACh concentration at the onset of AD is 30% of normal

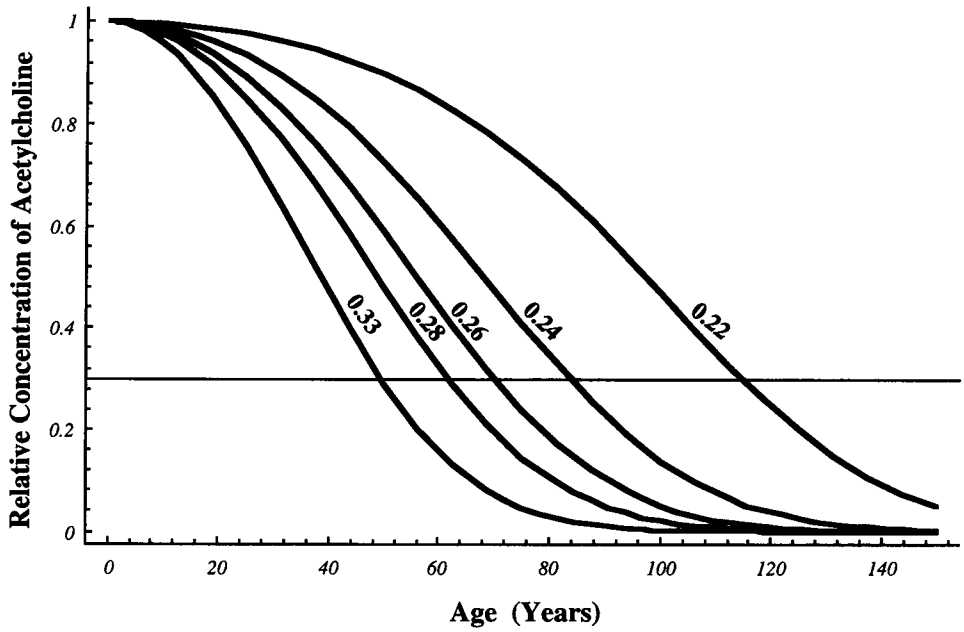

FIGURE 1 Calculated time course of the decrease in ACh concentration for several values of k_2, the rate of β-amyloid production. Each curve is labeled with the value of k_2 in units of nM/yr. Initial value of a = 50 nM; k_1 = 7.5 μM^{-1} $year^{-1}$; k_3 = 0.0042 $year^{-1}$; k_4 = 0.01 $year^{-1}$. The horizontal line, which corresponds to 30% of the initial ACh concentration, is a typical ACh concentration at the onset of AD.

(Tohgi et al., 1994). Accordingly, a horizontal line representing 30% of the initial ACh concentration is drawn in Fig. 1. The intersections of this line with the curves in Fig. 1 provide rough estimates of the predicted ages at which the ACh concentration decreases to the level characteristic of the onset of AD, and thus of the predicted age of onset of AD itself. This correspondence is based simply on the correlation between the loss of ACh and the onset of AD.

For the uppermost curve in Fig. 1, where k_2 = 0.22 nM/yr, the age of onset is ~114 years. Thus this curve pertains to an individual who is very unlikely to develop AD. At the other extreme in Fig. 1, where k_2 = 0.33 nM/yr, 50% higher than the value for the uppermost curve, the age of onset is ~50 years. This might correspond to an individual with Down's syndrome, because the presence of an extra 21st chromosome would be expected to cause a 50% increase in APP (Rumble et al., 1989), and hence a 50% increase in k_2. Indeed, it is well documented that individuals with Down's syndrome develop AD in middle age (Lai and Williams, 1989; Mann, 1988; Wisniewski et al., 1985).

The dependence of the age of onset of AD on the value of k_2 for the simplified model is shown in Fig. 2, where it can be seen that the age of onset declines rapidly for small increases in the value of k_2. For example, an increase in k_2 of 10% lowers the age of onset from 114 to 82, and an increase of 20% lowers it to 68.

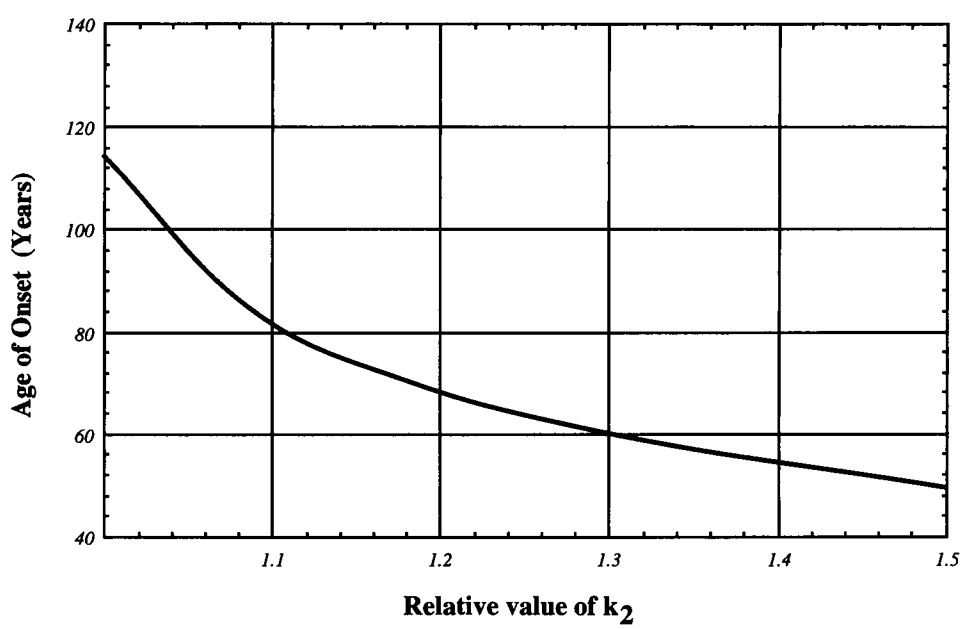

FIGURE 2 Dependence of calculated age of onset on k_2. Age of onset is determined by the intersection of each time course curve with the horizontal line in Fig. 1.

SIGNIFICANCE OF THE PREDICTIONS OF THE MATHEMATICAL MODEL

It is interesting that, on the basis of a relatively small amount of biological variability, the simplified model predicts a large variability in the age at which a dramatic loss of ACh occurs. The relation between this large variability and the large variability in the age of onset of AD itself may be fortuitous. For example, several parallel processes associated with AD may each involve positive feedback, which is key to the predictions shown in Fig. 1. This could also account for the correlation between decline in CSF ACh concentration and increasing severity of dementia (Davis et al., 1982; Tohgi et al., 1994). Alternatively, the large variability in age of onset may apply to both the decline in ACh concentration and the development of AD because of a causal relationship. ACh loss could be either a direct cause or an indirect cause of AD.

Although the hypothesis proposed in this paper does not depend on whether the loss of ACh leads to other characteristics of AD, it is interesting to consider how such a loss could cause further damage. One possibility is that decreased ACh could cause a loss of phosphatidylcholine, and that depletion of this phospholipid would compromise the properties of plasma membranes of cholinergic neurons, leading to neuronal death (Wurtman, 1992). Another possibility is that ACh is a neuronal trophic factor, analogous to the many other neurotransmitters that have been shown to act as trophic factors (Schwartz, 1992). There is also the possibility that the reduction in ACh concentration is indirectly responsible for neuronal death. This could occur by means of a reduction in NGF concentration, as suggested by the observation that NGF mRNA and NGF protein are up-regulated in the rat hippocampus by the activation of muscarinic receptors (Knipper et al., 1994). Thus a reduction in ACh concentration might be expected to cause down-regulation of NGF. Another possibility is that a reduction in ACh concentration could increase the concentration of β-amyloid by means of the positive feedback loop described above, but a β-amyloid-dependent process, such as calcium leakage (Arispe et al., 1993; Fukuyama et al., 1994), could be the proximate cause of neuronal death. A causal relation between loss of ACh and development of AD could also provide a rationale for the earlier onset of AD that has been found for individuals with apoE4, compared to individuals with other alleles of APOE (Strittmatter et al., 1993), because apoE4 is more effective in promoting β-pleated-sheet assembly (Castano et al., 1995). Such a conformational change would increase the membrane partition coefficient of β-amyloid and hence would increase the leakage of both choline and calcium.

The possibility that the loss of ACh is an important factor in the overall development of AD (termed the "cholinergic hypothesis" for AD) has been considered by many previous authors (cf. reviews by Bartus et al., 1982, and Winblad et al., 1993). One reason why interest in this hypothesis has waned in recent years is the relatively minor improvement obtained in clinical trials with either supplementary dietary choline or with acetylcholinesterase inhibitors such as tacrine or donepezil hydrochloride (Wurtman, 1994). According to the choline-leakage hypothesis, even if the loss of ACh were a basic cause of AD, it is unlikely that these agents would be very effective, because by the time symptoms appear, there is already a significant loss of ACh and a significant increase in membrane β-amyloid. Thus even if the treatments tended to increase the concentration of ACh, choline leakage would continue because of the relatively high concentration of β-amyloid already in the membranes. In this regard, the difficulty associated with administration of choline or acetylcholinesterase inhibitors after AD symptoms appear is somewhat analogous to the difficulty in baling out a leaky boat without repairing the leak. Whether choline or acetylcholinesterase inhibitors were administered simply to counteract the loss of ACh in AD or to counteract AD, itself, a more effective strategy would be to provide these agents before the leak is serious, i.e., before AD symptoms appear. Thus it might be reasonable to provide acetylcholinesterase inhibitors (or any other pharmacological agent believed to be efficacious in combating AD) to asymptomatic individuals who have a very high probability of developing AD, such as individuals with mutations known to cause AD or individuals with Down's syndrome.

CONCLUSION

The choline-leakage hypothesis described in this paper is consistent with a number of experimental observations, some of which are difficult to explain by other models. One goal of this presentation is to stimulate testing of the hypothesis. There are also several implications regarding therapeutic strategies that are inherent in the hypothesis. One implication is that it is crucial to start therapy at an early stage. Another is that it may be useful to seek agents that can compete with or interfere with the putative β-amyloid-induced leakage.

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