A Positive-Feedback Model for the Loss of Acetylcholine in Alzheimer's Disease

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ABSTRACT: We describe a two-component positive-feedback system that could account for the large reduction of acetylcholine that is characteristic of patients with Alzheimer's disease (AD). One component is β-amyloid–induced apoptosis of cholinergic cells, leading to a decrease in acetylcholine. The other component is an increase in the concentration of β-amyloid in response to a decrease in acetylcholine. We describe each mechanism with a differential equation, and then solve the two equations numerically. The solution provides a description of the time course of the reduction of acetylcholine in AD patients that is consistent with epidemiological data. This model may also provide an explanation for the significant, but lesser, decrease of other neurotransmitters that is characteristic of AD.

INTRODUCTION

Although there is strong evidence that β-amyloid plays an important role in Alzheimer's disease (AD),[1,2] it is not yet clear what that role is. There are several mechanisms whereby β-amyloid could affect the concentration of neurotransmitters. In this paper, we consider one of these mechanisms, β-amyloid-induced apoptosis,[1,3] as part of a positive feedback loop that would result in a significant loss of acetylcholine (ACh).

We previously considered β-amyloid-induced leakage of choline[4,5] out of cholinergic neurons as a possible mechanism for the reduced ACh concentration that correlates with AD.[6,7] Since the choline concentration is rate-limiting for the production of ACh, choline leakage would cause a reduction in ACh concentration. We then modeled the positive feedback[8] that would be generated as a result of this reduction of ACh concentration, whose basic cause is an increase in β-amyloid concentration, and the increase in β-amyloid concentration that has been shown to be caused by the reduction of ACh concentration. One difficulty with our previous approach is that there is not yet any experimental information regarding the magnitude of choline leakage in a relevant system. Accordingly, we had to use an arbitrary value for the

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magnitude of choline leakage in our calculations. Interestingly, with an appropriate choice for this value, we were able to obtain results that are consistent with epidemiological data, thus demonstrating the potential value of a positive feedback model involving loss of ACh and increase of β-amyloid.

We then sought an alternative mechanism for the reduction of ACh concentration by β-amyloid for which there is experimental data on the magnitude of the effect. Interestingly, such a mechanism has been reported. It had been shown previously that β-amyloid induces apoptosis in cultured neurons.[1] It had also been shown that β-amyloid is much more toxic to PC12 cell mutants that express p75 than to PC12 mutants that do not express p75 and that this toxicity is significantly enhanced by transfecting the latter with p75.[9] Recent experiments have shown that both β-amyloid and p75 have a role in causing cell death.[3] These experiments not only demonstrated directly that binding of β-amyloid to p75 receptors causes apoptosis, but also provided data on the magnitude of the effect.[3] Accordingly, the mechanism we consider in this paper is β-amyloid–induced apoptosis.

In addition to a large decrease in the concentration of ACh, AD patients also experience significant, but lesser, decreases in the concentrations of other neurotransmitters.[10] A possible explanation for this is that β-amyloid-induced apoptosis affects all secreting cells that contain p75. As a result of the death of these secreting cells, there would be a decrease in the concentration of the various neurotransmitters that would be proportional to the relative concentration of p75 in cells secreting each neurotransmitter. ACh concentration would be most affected because the relative concentration of p75 is highest in cholinergic neurons of the basal forebrain.[11,12]

Our treatment includes a mathematical description of the binding of β-amyloid to p75 and the resultant apoptosis, but does not address the intermediate steps between binding and apoptosis. Although the details of these intermediate steps are not yet known, it is likely that free radicals play a crucial role. Evidence that binding to p75 could lead to formation of free radicals was obtained in experiments showing colocalization of p75 and nitric oxide synthase in rat brains.[13] Evidence that free radicals can cause apoptosis has been found both directly and indirectly. Ever since the pioneering work of Gerschman, Gilbert, and their collaborators,[14] it has been clear that free radicals can have a devastating effect on cell viability. Recent work shows that free radicals can cause apoptosis in both neurons[15] and glial cells.[16] Furthermore, antioxidants that destroy excess free radicals, such as the bcl-2 gene product, have been shown to prevent apoptosis.[17]

DESCRIPTION OF POSITIVE FEEDBACK MODEL

β-amyloid, which is a product of the breakdown of the amyloid precursor protein (APP), binds to p75 in cholinergic cells, leading to apoptosis of these cells[3] and a consequent decrease of ACh. A decrease in ACh concentration has two effects relevant to the concentration of β-amyloid. It causes an increase in the synthesis of APP in cerebral cortex, as reflected by increased levels of APP mRNA,[18] and it favors the processing of APP by means of the β-amyloid pathway.[19,20] 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 increase in

apoptosis 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 increase in the concentration of β-amyloid and a significant decrease in the concentration of ACh. The increase in β-amyloid would also cause a decrease in the concentration of other neurotransmitters.

MATHEMATICAL DESCRIPTION

Let a denote the concentration of ACh; b, the concentration of β-amyloid; p, the concentration of unbound p75 receptors in cholinergic neurons; p_t, the total concentration of p75 receptors in cholinergic neurons; and let p_b denote the concentration of p75 bound to β-amyloid.

The loss of cholinergic neurons by apoptosis is caused by the binding of β-amyloid to p75 receptors. The rate of apoptosis, which is the rate at which cholinergic cells are lost, is proportional to p_b. Assuming first-order binding of β-amyloid to p75,

$$\frac{da}{dt} = -k' p_b = -\frac{k' p b}{k_b}, \tag{1}$$

where k_b is the dissociation constant for binding of β-amyloid to p75 and k' is a constant of proportionality. The concentration of available receptors, p, can be expressed in terms of the total concentration of receptors, p_t:

$$p = \frac{p_t}{1 + b/k_b}. \tag{2}$$

Combining **(1)** and **(2)**,

$$\frac{da}{dt} = \frac{-k' p_t b}{k_b + b}. \tag{3}$$

Thus, a and p_t are each proportional to the number of cholinergic cells, and hence are proportional to each other:

$$p_t = k'' a. \tag{4}$$

Combining **(3)** and **(4)**,

$$\frac{da}{dt} = \frac{-k' k'' a b}{k_b + b}. \tag{5}$$

In order to simplify comparison of this equation for loss of ACh with the comparable equation in our previous model, let $k' k'' = k_1 k_b$. Then

$$\frac{da}{dt} = \frac{-k_1 k_b a b}{k_b + b}. \tag{6}$$

Equation **(6)**, which describes the loss of ACh as a result of apoptosis of cholinergic neurons, is one of a pair of equations that represent the positive feedback model. The

other equation describes the increase in β-amyloid in response to a decrease in ACh. As indicated previously,[8] the increase in β-amyloid can be described by the following equation:

$$\frac{db}{dt} = k_2 - k_3 a - k_4 b. \quad \textbf{(7)}$$

Equation **(7)** describes the increase in the concentration of β-amyloid resulting from its formation from the breakdown of APP (represented by the term k_2), the effect of ACh on its rate of formation[18,19,20] (represented by the term $-k_3 a$), and the decrease in the concentration of β-amyloid resulting from enzymatic breakdown and absorption into neuronal membranes (represented by the term $-k_4 b$).

The simultaneous solution of Equations **(6)** and **(7)** provides a description of the time course of the decline in ACh concentration and the increase in β-amyloid concentration. These equations were solved numerically by use of the Mathematica program. Two of the constants in these equations can be estimated from the data presented by Yaar *et al*.[3] The dissociation constant k_b was measured directly, and is about 0.025 μM. To calculate k_1, consider Equation **(6)** for small values of b:

$$\frac{da/dt}{a} = -k_1 b.$$

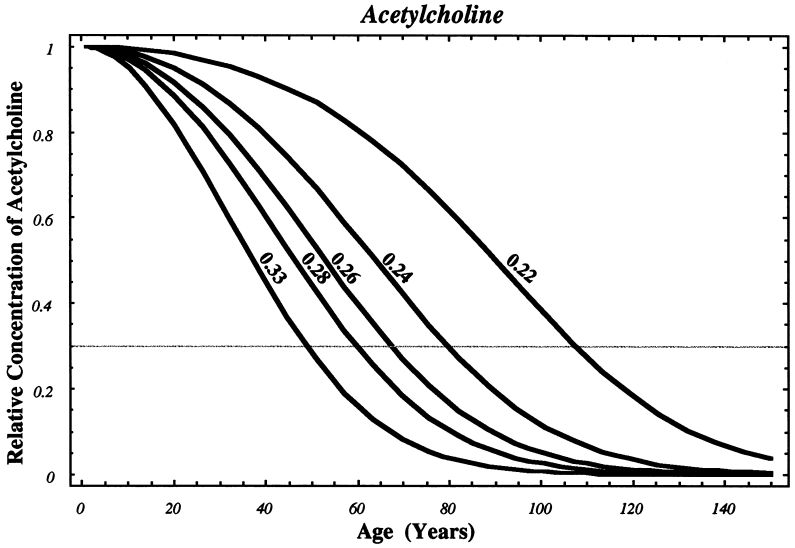

FIGURE 1. Calculated time course of the decrease in ACh concentration for several values of k_2, the rate of β-amyloid production. Each curve is labeled with the value of k_2 in units of nM/yr. Initial value of a = 50 nM; k_1 = 9.0 μM^{-1} yr^{-1}; k_3 = 0.0042 yr^{-1}; k_4 = 0.01 yr^{-1}. The horizontal line, which corresponds to 30% of the initial ACh concentration, is a typical ACh concentration at the onset of AD.

For this condition, the fractional rate of decrease of a is proportional to b. Thus, the value of k_1 can be estimated from the limiting slope for small b of the curve showing the rate of decrease of a as a function of b. From the data on melanocytes by Yaar *et al.*[3], we estimate the limiting slope to be about $-9\ \mu M^{-1}\ yr^{-1}$. Thus, $k_1 = 9\ \mu M^{-1}\ yr^{-1}$. Interestingly, this experimentally based value for k_1 is quite close to the value for the corresponding constant k_1 that we chose in our previous model[8] to give results consistent with epidemiological data.

The assumed initial value for b is zero. Also, the same initial value of a and the same values for k_3 and k_4 were used in this calculation as in our previous model. These values are indicated in the legend of FIGURE 1. Since the numerical solutions were found to be very sensitive to the value of k_2 (the rate of β-amyloid production that would occur if no ACh or β-amyloid were present), solutions were obtained for a range of k_2 values.

SIGNIFICANCE OF THE PREDICTIONS OF THE POSITIVE FEEDBACK MODEL

The time course of the decline in ACh concentration according to the positive feedback model described above is shown in FIGURE 1. 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[6,7] and that a reasonable estimate of the ACh concentration at the onset of AD is 30% of normal.[7] Accordingly, a horizontal line representing 30% of the initial ACh concentration is drawn in FIGURE 1. The intersections of this line with the curves in FIGURE 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 ages 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 FIGURE 1, where $k_2 = 0.22$ nM/yr, the age of onset is about 107 years. Thus, this curve pertains to an individual who is very unlikely to develop AD. At the other extreme in FIGURE 1, where $k_2 = 0.33$ nM/yr, 50% higher than the value for the uppermost curve, the age of onset is about 49 years. This might correspond to an individual with Down's syndrome, since the presence of an extra 21st chromosome would be expected to cause a 50% increase in APP,[21] and hence a 50% increase in k_2. Indeed, it is well documented that although AD is usually a disease of old age, individuals with Down's syndrome develop AD in middle age.[22–24]

The dependence of the age of onset of AD on the value of k_2 for the positive feedback model described above is shown in FIGURE 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 107 to 78 and an increase of 20% lowers it to 65.

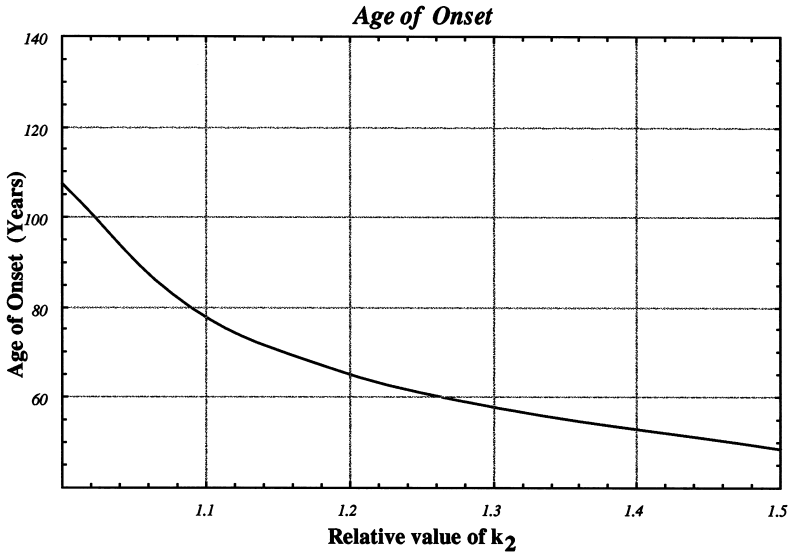

FIGURE 2. Dependence of calculated age of onset on k_2. Age of onset is determined by the intersection of each time course curve with the horizontal line in FIGURE 1.

DISCUSSION

Positive feedback between a decrease in ACh concentration and an increase in β-amyloid concentration requires two interacting mechanisms—one mechanism whereby β-amyloid causes a loss of ACh and one mechanism whereby ACh loss causes an increase in β-amyloid. In the present paper, we invoke the same mechanism for the increase in β-amyloid that we used in a previous attempt to model this type of positive feedback.[8] Therefore, one of the differential equations is the same in both treatments. For the loss of ACh, however, we invoke a completely different mechanism in this paper than we did in our previous treatment. Here we consider β-amyloid–induced apoptosis, whereas we previously considered β-amyloid–induced choline leakage. Although these two mechanisms are very different, the equations describing them are rather similar. The main difference in the form of the two equations is that apoptosis is based on binding, and hence the amount of apoptosis tends to saturate at relatively high concentrations of β-amyloid. By contrast, choline leakage was assumed to be linear, since the experiments describing β-amyloid–induced choline leakage showed a linear dependence of leakage on β-amyloid concentration.

In view of the general similarity between the two sets of equations, it is not surprising that the overall results shown in FIGURES 1 and 2 are rather similar to the equivalent figures presented in the previous treatment. In both models, FIGURES 1 and 2 provide a rationale for the well-known observations that AD is a disease of old age and that there is a wide range in the age of onset. A major advantage of the

present treatment is that we can use experimental data to estimate the constants that describe the magnitude of the apoptosis leading to the loss of ACh. The fact that FIGURE 1 is consistent with epidemiological data on AD indicates that the rate of apoptosis that has been found experimentally can account for the observed loss of ACh in AD.

The present treatment can also explain several important observations regarding the loss of neurotransmitters in AD. The neurotransmitter most affected in AD is ACh.[10] However, not all cholinergic neurons are affected. According to the present treatment, only those neurons expressing p75 would undergo apoptosis. Interestingly, cholinergic neurons of the basal forebrain, which are particularly predisposed to degeneration in AD,[25,26] express the highest levels of p75 of any neurons tested,[27] whereas cholinergic neurons of the pontomesencephelon, which do not undergo degeneration in AD,[28] do not express p75.[27] Thus, our model provides a rationale for the spatial variability of ACh loss.

Although the positive feedback loop we have described affects only ACh, the model predicts that the increased β-amyloid concentration brought about by the positive feedback would also increase apoptosis of any neurons that contain p75. As a result, there would be a decrease in the concentration of other neurotransmitters that is proportional to the relative concentration of p75 in the relevant secreting cells. Although earlier experiments indicated that p75 was limited to certain cholinergic neurons,[29] more sensitive methods have demonstrated that lower concentrations of p75 are present in many areas of both the rat brain[30] and the human brain,[12] including the hippocampus, caudate, cerebellum, and olfactory bulb. Since p75 mRNA was found in areas of the brain that do not contain, and are not innervated by, cholinergic neurons, the model also provides a rationale for the observed decrease in concentration of neurotransmitters other than ACh.[10]

Finally, a positive-feedback model can provide a possible rationale for the fact that acetylcholinesterase inhibitors have had only limited success as therapeutic agents in AD. Because of the putative positive feedback, the loss of ACh would cause an increase in β-amyloid and a consequent loss of more ACh. Thus, even if the acetylcholinesterase inhibitors do increase the ACh concentration, they would be working in a system with a relatively large concentration of β-amyloid and with increasing ACh deficits. For a positive-feedback system, the best way to cope with this difficulty is to start treatment at a very early stage of the disease.[8] Since it is currently difficult to detect AD at a very early stage, a first step might be to provide acetylcholinesterase inhibitors or agents that prevent β-amyloid from binding to p75 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.

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