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Alzheimer's Disease Brain: Alterations in RNA Levels and in a Ribonuclease-Inhibitor Complex

Abstract. *A macromolecular alteration occurs at the posttranscriptional level in the Alzheimer's disease (AD) brain. Compared with age-matched controls, total cellular RNA and polyadenylated RNA were substantially reduced in the AD cortex with many neuritic plaques and neurofibrillary tangles. RNA changes are associated with a significant increase in alkaline ribonuclease activity due to an abnormality in the ribonuclease-inhibitor complex. The decrease in protein synthesis in the AD brain, previously observed in patients severely affected with AD, and in translation systems in vitro with AD cortical messenger RNA, may be partly related to an enzyme-inhibitor alteration that affects RNA levels and activity. Decreased protein synthesis therefore may contribute to the characteristic decline in certain neurotransmitter enzymes and to the loss of neurons in the AD brain.*

The Alzheimer's disease (AD) brain is characterized by loss of specific neurons, and the degree of dementia can be correlated with cerebral neuronal degeneration involving neuritic (senile) plaques and neurofibrillary tangles and a decline in choline acetyltransferase activity (*1*). Although it is unlikely that the dramatic intra- and extracellular pathology occurs by processes unrelated to transcriptional or translational control mechanisms (or both), data concerning macromolecular alterations that may be relevant to the etiology of AD are limited. Our initial studies showing a decline in protein synthesis directed by AD brain messenger RNA (mRNA) in vitro (*2*) were independently confirmed by positron emission tomography studies that showed decreased incorporation of methionine into brain protein of patients afflicted with AD (*3*). Various factors may contribute to the decline in protein synthesis in affected regions of the AD brain. We have examined one of these factors, namely that the severely affected AD cortex has decreased levels of ribosomal RNA (rRNA) and mRNA due to a posttranscriptional alteration at the level of RNA degradation (*4*).

Brain and other mammalian tissues contain a major "alkaline" ribonuclease, which is active at *p*H 7.2 to 8.0 (*5–7*). Unlike acidic ribonuclease, which is localized in lysosomes along with other acid hydrolases (*8*), the alkaline enzyme in brain homogenates is primarily localized in the soluble fraction, and to a lesser extent in nuclei and microsomes, and small amounts can be detected in other subcellular fractions (*9*); higher activities are present in neuronal rather than glial nuclei (*10*). The activity of alkaline ribonuclease is restricted by the presence of a bound inhibitor (*6*), a 50- to 60-kD protein (*11*), that is present in higher concentrations in brain than other organs (*6, 12*). Inhibitor from brain and liver supernatants has been used to stabilize polysome preparations (*13*). Because of the presence of inhibitor in tissue homogenates, the ribonuclease activity is relatively low and is referred to as free ribonuclease. When the inhibitor is inactivated by sulfhydryl-blocking reagents, such as *p*-chloromecuribenzoate (PCMB) (*6*), the activity increases and is referred to as total alkaline ribonuclease.

Control samples of cerebral cortex were obtained at autopsy from four individuals that had died with no history of dementia or other neuropsychiatric disease; the mean age at death was 72 ± 5.6 years and the postmortem interval was 8.7 ± 1.8 hours. On the basis of the clinical history of dementia and the histopathologic identification of both neuritic plaques and neurofibrillary tangles, the diagnosis of AD was established in six patients with a mean age at death of 70.8 ± 2.9 years; the interval between death and autopsy was 11.9 ± 2.9 hours [(*14*) and the legend to Fig. 1].

Total cellular RNA, consisting predominantly of ribosomal RNA, was prepared from the foregoing cortical specimens that had been preserved at −70°C and not thawed prior to extraction (*15*). The average yield from the control brain specimens was 100 ± 7.7 μg of RNA per gram of tissue as compared to 54.7 ± 9.5 μg/g from AD specimens (Fig. 1). The difference is statistically significant [$t(10) = 3.69$; $P < 0.01$] and represents a 45.3 percent reduction in the yield of RNA extracted from brain tissue containing both plaques and tangles as compared with controls. The relative decrease in AD RNA is consistent with measurements obtained by histologic staining of postmortem brain (*16*).

The foregoing total cellular RNA samples were used to prepare poly(A)$^+$ (polyadenylated) RNA by oligo(dT)-cellulose (deoxythymidylate) column chromatography (*17*). These described procedures applied to both control and AD postmortem brains allow the preparation of poly(A)$^+$ RNA that retains mRNA activity during in vitro protein synthesis (*2, 15*). The average yield of poly(A)$^+$ RNA from control brains was 2.8 ± 0.4 μg/g; the average yield of poly(A)$^+$ RNA from the series of AD specimens was 1.0 ± 0.2 μg/g, a significant relative reduction [64.3 percent decrease, $t(10) = 3.85$, $P < 0.01$] compared with controls. When data from the foregoing brains were analyzed together with data from a larger series of control and AD specimens a significant correlation could not be established between the yield of total cellular RNA [$r = -0.32$; $t(14) = 1.16$; $P > 0.2$] or of poly(A)$^+$ RNA [$r = -0.47$; $t(14) = 1.96$; $P > 0.05$] and the postmortem interval in the range of 3.5 to 25 hours.

Cortical specimens adjacent to those taken for RNA preparations were used to measure the free and total alkaline ribonuclease activity at *p*H 7.5 (Fig. 2); like acidic ribonuclease, alkaline ribonuclease retains its activity in previously frozen tissue (*20*). Denatured mammalian [^{3}H]rRNA was added to serve as a substrate. The mean free alkaline ribonuclease activity (per milligram of tissue) was 39.3 ± 6.2 units in control specimens (Fig. 2). Among AD brains the mean free ribonuclease activity was 73.6 ± 10.5 units, which represented a significant increase (87.3 percent) over control values [$t(10) = 2.80$; $P < 0.05$].

In the presence of PCMB (1 m*M*) all control cortical samples showed a substantial increase (133.1 percent) in alkaline ribonuclease which ranged from 35 to 62 units with a mean increase of 52.3 ± 6.2 units (Fig. 2B). By contrast, the mean increase in alkaline ribonucle-

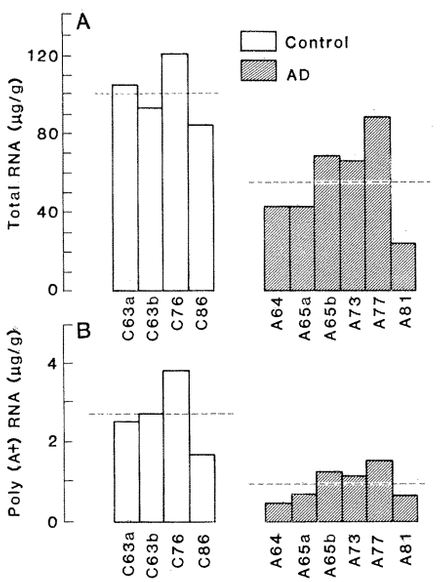
ase from AD samples was only 0.47 ± 5.1 units, with one sample exhibiting moderate stimulation (Fig. 2B). The differential effect of PCMB on control and AD samples was also observed after the foregoing cortical homogenates were assayed again, this time with *E. coli* [^{3}H]rRNA as substrate (control = 53.6 ± 19.8 units; AD = 0.08 ± 3.3). The difference in postmortem interval appeared inadequate to explain the differences between the two groups. For example, C86 with the longest interval of control specimens retained significantly more inhibitor activity than AD cortices with shorter intervals (A64, A73, A77, and A81) (Figs. 1 and 2).

The extensive differences in the alkaline ribonuclease system between control and AD specimens were not paralleled by similar changes in acid ribonuclease. Acid ribonuclease activity, assayed at *p*H 6.0 (*18*), was higher in AD specimens: 81.3 ± 2.4 units as compared to the control value of 70.7 ± 3.3; the difference represents an increase in AD of 15.0 percent [$t(8) = 2.62$, $P < 0.05$]. In the presence of PCMB, controls were increased an average of 11.6 ± 3.9 units and AD samples by 5.1 ± 2.9 units. The limited effect of inhibitor on lysosomal acid ribonuclease has been established (*6, 19*).

Results of our studies are consistent with the hypothesis that the relatively lower levels of RNA in the AD cortex are related, in part, to a posttranscriptional control mechanism, that is, decreased inhibition of ribonuclease resulting in increased degradation of RNA. The levels of mammalian cytoplasmic alkaline ribonuclease inhibitor vary with the metabolic state of tissue (*7, 20*). Cells with high rates of both RNA accumulation and protein synthesis have relatively high ratios of inhibitor to alkaline ribonuclease and this ratio is decreased in tissues with increased rates of catabolism or with lower rates of protein synthesis (*7, 20*).

In this regard, positron emission tomography studies have shown a 65 percent decrease (or more) in brain protein synthesis in patients with severe AD as compared with controls (*3*). In addition, poly(A)$^+$ mRNA prepared from the postmortem AD cortex retains less activity than that from the control during protein synthesis in vitro (*2*). Thus downward regulation of protein synthesis in cortical and subcortical areas may profoundly influence neuronal functioning, and may directly affect the levels of neurotransmitter enzymes as well as the survival of neurons.

Our study has been made with neocortical samples, rich in both plaques and tangles, that were derived from frozen postmortem brains obtained from patients dying with AD. The observation that one in six AD cases had either a normal RNA yield or alkaline ribonuclease activity suggests that quantitative variations in these parameters may be found among brains selected by clinicopathologic criteria other than those we used here; and regional differences may be expected to exist. At present it is not clear whether the decreased brain RNA and protein synthesis are manifestations of AD or are predisposing factors. However, the observation that an altered ribonuclease-inhibitor complex can be demonstrated in the cortices of severely demented patients suggests the usefulness of examining additional macromolecular control mechanisms that may be involved in the pathogenesis of AD.



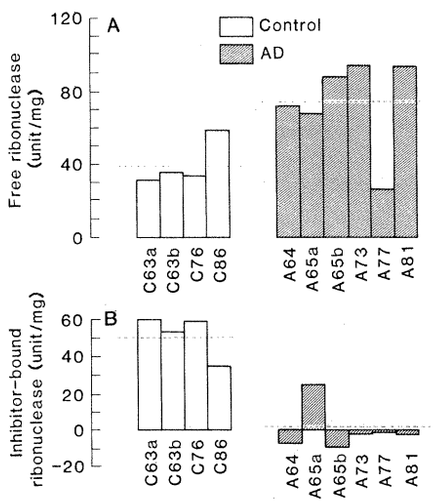


Fig. 1 (left). Yield of total cellular RNA (panel A) and of poly(A)$^+$ RNA (panel B). Control brains are indicated by prefix C and AD brains by prefix A; following these designations are ages at death. The postmortem intervals are as follows: C63a, 6 hours; C63b, 8 hours; C76, 7 hours; C86, 14 hours; A64, 12 hours; A65a, 9.5 hours; A65b, 25 hours; A73, 4 hours; A77, 12 hours; and A81, 9 hours. AD specimens were independently rated as typically +3 to +4 for both plaques and tangles (*14*). RNA was prepared from frontal, parietal, and temporal cortical specimens (*15, 17*). RNA yields, determined by optical absorbance (A_{260}), are expressed in micrograms per gram (wet weight) of cortex. Horizontal lines indicate mean values for each group of RNA determinations. Fig. 2. (A) Free brain alkaline ribonuclease activity in the absence of PCMB. (B) The inhibitor-bound ribonuclease activity expressed as the difference between the total activity in the presence of ImM PCMB (*6*) and the free activity measured in the absence of PCMB. Horizontal lines indicate mean values for each group of ribonuclease determinations (see text). Frozen samples were disrupted by homogenization in water and diluted with 0.06*M* citrate-phosphate buffer, *p*H 7.5 (*5*). EDTA (0.5 m*M*) was added to prevent contamination of exogenous RNA by divalent cations and release of latent ribonuclease activity (*6*). The incubation mixture contained 2 mg (wet weight) of cortical homogenate (see legend to Fig. 1), 4.3 μg of calf liver RNA (PL Biochemicals, No. 2506), and 12,656 dis/min of HeLa cell [^{3}H]rRNA (Bethesda Research Laboratories, No. 5601 SA) in a total volume of 0.5 ml. Exogenous rRNA was denatured at 70°C for 5 minutes prior to use in assay mixtures. The mixtures were incubated at 37°C for 60 minutes. The reaction was terminated by 50 μg of rRNA and 10 percent trichloroacetic acid containing 0.1*M* sodium pyrophosphate. RNA was recovered on nitrocellulose paper (Millipore) by filtration and washed three times with 5 percent trichloroacetic acid containing 0.05*M* sodium pyrophosphate and once with 70 percent ethanol. The dried filters were treated with 5 ml of Bray's solution, and the radioactivity was counted (Packard spectrometer, 15 percent efficiency). To correct for nonspecific degradation of RNA, reaction mixtures containing the foregoing additives with the exception of brain homogenate were incubated in parallel; the radioactivity in the acid precipitable fraction thus determined represented no hydrolysis (0 percent). Under the assay conditions there was a linear relationship between the amount of added brain homogenate in the range of 0.5 to 6 mg (wet weight) and the degradation of RNA. One unit of activity is defined as 1 percent hydrolysis of exogenous [^{3}H]RNA per 60 minutes.

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15. Total cellular RNA was prepared by method IV described in E. Sajdel-Sulkowska, J. F. Coughlin, C. A. Marotta, *J. Neurochem.* **40**, 670 (1983).
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17. Poly(A)$^+$ RNA was prepared by the one-step oligo(dT)-cellulose column chromatography (*15*). The RNA yields obtained after precipitation with ethanol (measured by optical absorbance at A_{260}) are indicated in the text and in Fig. 1B. The RNA yields of column eluates, prior to precipitation, are as follows: controls, 3.9 ± 0.8 μg/g; AD, 1.6 ± 0.3 μg/g. The latter value represented a significant relative reduction [59.7 percent decrease, $t(10) = 2.78$, $P < 0.05$].
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Mating and Pregnancy Can Occur in Genetically Hypogonadal Mice with Preoptic Area Brain Grafts

Abstract. *Adult female hypogonadal mice, in whom hypogonadism is secondary to a genetic deficiency in hypothalamic gonadotropin-releasing hormone (GnRH), are infertile. Mating, pregnancy, and delivery of healthy litters were achieved after transplantation of normal fetal preoptic area tissue, a major site of GnRH-containing cell bodies, into the third ventricle of adult female hypogonadal mice. Immunocytochemistry revealed GnRH-containing neurons in the grafts and GnRH-containing processes extending to the lateral median eminence of the host brains.*

Infertility in the hypogonadal mouse is secondary to a genetic deficiency of hypothalamic gonadotropin-releasing hormone (GnRH) (*1*). Affected animals have infantile gonads, undeveloped secondary sexual tissue, and low concentrations of pituitary and plasma gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)]. A similar condition in humans, familial gonadotropin deficiency (*2*), appears to be inherited as an autosomal recessive trait, as is the hypogonadal defect (*1*). GnRH injections stimulate LH and FSH production in affected mice (*3*) and humans (*4*). In normal adult rodents, GnRH-containing cell bodies are present in the preoptic area (POA) of the brain. Several diverse neurochemical deficits related to genetic or surgical lesions have been alleviated by grafts of brain tissue (*5*). We previously reported that grafts of normal fetal POA tissue into the third ventricle in adult male (*6*) or female (*7*) hypogonadal mice corrected many of their endocrine defects. In both sexes significant increases in pituitary and plasma LH and FSH were associated with increased weights of gonadal and accessory sex organs. However, after vaginal opening, the cells in daily vaginal smears were cornified, indicating constant secretion of estrogen rather than the characteristic 4- to 6-day ovulatory cycle of normal mice. Since no corpora lutea were seen in the ovaries, we had no evidence that the females were capable of ovulation.

We now report that ten adult hypogonadal females with POA grafts (*8*) mated when paired overnight with a normal male. Seven of these females became pregnant and six delivered healthy litters. Histological and immunocytochemical analyses of the brains of these females confirmed that all had received GnRH-containing grafts in the third ventricle.

In view of the known sexual dimorphism of the POA region (*9*), we sought to determine whether the sex of the donor was important in the success of the POA transplant. Therefore, five of the hypogonadal females received grafts of POA tissue from female fetuses and five received grafts from male fetuses. Vaginal opening, induced by estrogen secretion from the stimulated ovaries, occurred 16 to 40 days after transplantation, and the females entered constant vaginal estrus.

Ten weeks after transplantation the hypogonadal females were each paired with a normal male overnight. In the morning nine of the females were found to have vaginal plugs, a sign of successful copulation. The remaining female was paired with one of the proven males a second night, and she too mated. The females were weighed every 3 to 4 days to check for pregnancy; seven showed weight gains. Six females delivered live litters of four to seven pups each. One female died 11 days after mating, but an autopsy showed that she held three embryos.

When the pups were 1 month old, blood was collected under ether anesthesia from the nine surviving dams. The plasma was stored at −20°C for later radioimmunoassay of LH and FSH (*10*). The dams' brains were then prepared for immunocytochemical identification of GnRH. Four animals were perfused (*11*) for subsequent sectioning of brain tissue with a Vibratome. The remaining animals were decapitated and their brains were processed for paraffin sectioning (*12*); pituitaries were frozen for subsequent radioimmunoassay of LH and FSH. The ovaries and uteri of all animals were removed and weighed.

Table 1 gives mean concentrations of pituitary and plasma LH and FSH in hypogonadal females with and without POA grafts and in normal homozygous mice of the same strain. Gonadotropin concentrations in hypogonadal females with grafts were in the normal range. Similarly, ovarian and uterine weights in hypogonadal females with POA grafts (12.4 ± 1.6 and 136.5 ± 15.1 mg, respectively; means ± standard errors) were comparable to those of the normal adults (12.5 ± 1.0 and 161.0 ± 31.3 mg) and vastly greater than in the untreated hypogonadal adults (2.8 ± 0.6 and 12.2 ± 1.6 mg).

The antiserum used for immunocytochemical demonstration of GnRH visualizes these peptidergic neuron cell bodies in normal mouse brain without requiring treatment of the animal with colchicine, and the reactive cells and fibers in this species have a distribution similar to that in rat brain (*13*). In normal mouse brain many GnRH-containing cell bodies are present in the POA-septal region, and a major projection of reactive fibers is observed in the lateral median eminence. With the antiserum no reactive cells or