Apolipoprotein E ε4 Allele and Pupillary Response to Tropicamide

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Objective: This study was designed to investigate in cognitively normal subjects the possible association between hypersensitive pupil dilation responses to the cholinergic antagonist tropicamide and the presence of the apolipoprotein E ε4 (APOE4) allele, a well-defined genetic risk factor for Alzheimer's disease. Method: The authors measured tropicamide-induced changes in pupil area in 44 cognitively normal Japanese subjects with and without the APOE4 allele. Results: The subjects with the APOE4 allele had significantly greater increases in pupil area than the others. The significant correlation of changes in pupil area with age for the subjects with the APOE4 allele was lacking for those without this allele. Conclusions: The results suggest that a hypersensitive pupil dilation response to tropicamide is already present in cognitively normal individuals with the APOE4 allele. This association also suggests the potential involvement of APOE4 in the mechanism of pupil dilation.

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Hypersensitive responses of the pupil to the cholinergic agonist pilocarpine and the antagonist tropicamide have been reported in patients with Alzheimer's disease (1, 2). Scinto et al. (1) proposed using these changes in postdrug pupil size in the diagnosis of Alzheimer's disease, because the increases in pupil size of patients with Alzheimer's disease are so marked that they hardly overlap normal subjects' responses. However, there are researchers who have failed to confirm hypersensitive pupil response to tropicamide in patients with Alzheimer's disease (3).

Studies have also revealed the strong association of apolipoprotein E (APOE) with sporadic and familial late-onset Alzheimer's disease; the frequency of the apolipoprotein E ε4 (APOE4) allele is markedly higher in patients with Alzheimer's disease (4). If pupillary hypersensitivity to anticholinergic agents is related to the Alzheimer's disease process, the pupil dilation response to a cholinergic antagonist may be influenced by the presence of the APOE4 allele in cognitively normal subjects as well as in patients with Alzheimer's disease. To test this hypothesis, we investigated pupillary responses to dilute tropicamide in healthy, cognitively normal Japanese subjects.

METHOD

This study was approved by the Ethics Committee of the Japanese National Institute on Alcoholism. Written informed consent was obtained from all subjects after we had carefully explained the details of the study.

The subjects were 44 healthy Japanese volunteers (20 male and 24 female) whose mean age was 56 years (SD=10, range=37–77) and who had brown eyes free of ocular pathology. The Mini-Mental State examination (5) showed that none of the subjects had signs of cognitive impairment (mean score=29.7, SD=0.6, range=28–30). After instruction in the experimental procedure, the subjects were placed prone in a semidarkened room and given time (more than 10 minutes) for their eyes to accommodate. The baseline resting pupil area of each eye was measured twice, 2 minutes apart, by an Iriscorder C2514 (Hamamatsu Photonics, Hamamatsu, Japan).

After obtaining baseline data, we administered one drop of 0.01% tropicamide to each right eye and one drop of a control solution (0.9% sodium chloride) to each left eye. For each right eye, we recorded pupil areas after 10, 30, and 50 minutes, using the arithmetic averages of the two pupil measurements (1 minute before and 1 minute after the scheduled time) for analyses. Left eye measurements were done once at each scheduled time.

To assess the reliability of the pupillary response measure, we repeated the test procedure more than a week after the original measurement in five subjects. The results gave almost the same measurements as the original ones in each subject (data not shown).

APOE genotyping was performed by the method of Wenham and co-workers (6). The pupillary response measurements were done without knowledge of APOE genotype. Using repeated measures analysis of variance, we tested the differences between the percent changes in pupil area at the scheduled times for the subjects with and without the APOE4 allele. Correlations between age and the changes in pupil area were examined with the use of Pearson's correlation coefficients.

RESULTS

Among these 44 subjects, the 16 with the APOE genotypes 3/4 and 4/4 became the APOE4 group; the 28 with 2/3 and 3/3 genotypes, the non-APOE4 group. The groups did not differ significantly in age (for the APOE4 group, mean=56 years, SD=12; for the non-APOE4 group, mean=56 years, SD=9).

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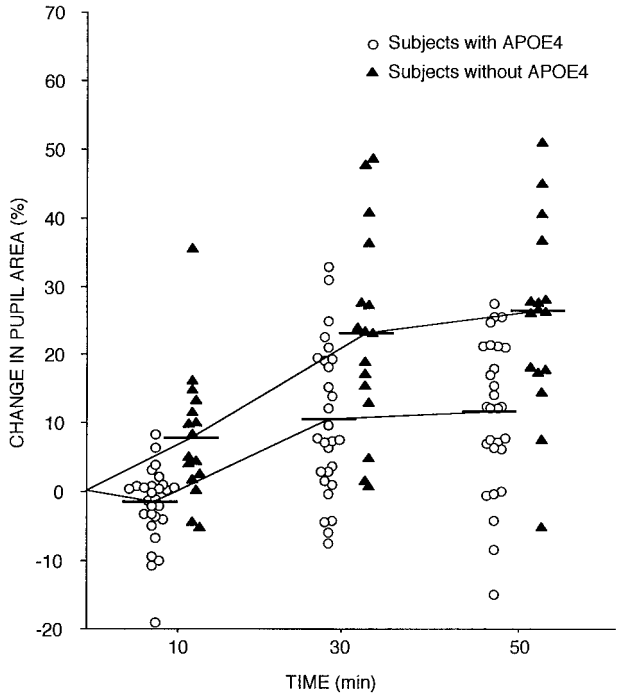
FIGURE 1. Percent Change in Area of the Pupil 10, 30, and 50 Minutes After Tropicamide Treatment in Subjects With the APOE4 Allele (N=16) and Without the APOE4 Allele (N=28)[a]



[a]Each bar represents the mean value for that genotype group at the 10-, 30-, and 50-minute time points. There was a significant difference between the two groups (repeated measures ANOVA, main effect for group: F=15.62, df=1, 42, p<0.0001).

The mean percent changes in pupil size 50 minutes after administration of the drug increased across APOE genotypes in this order: for 2/3, 7.7%; for 3/3, 12.2%; for 3/4, 23.6%; and for 4/4, 36.2%. Similar trends were observed at 10 and 30 minutes. The mean percent changes were significantly greater for the APOE4 group than for the non-APOE4 group over the course of time (figure 1).

The tropicamide-induced percent changes in pupil area appeared to increase with age for all subjects (data not shown); however, that tendency is attributable to the significant correlation between age and percent change in the APOE4 group at 30 minutes (r=0.58, df=14, p=0.02) and at 50 minutes (r=0.57, df=14, p=0.02). No such correlation was observed for the non-APOE4 group (30 minutes: r=0.15, df=26, p=0.45; 50 minutes: r=0.24, df=26, p=0.23).

DISCUSSION

In this study, individuals with the APOE4 allele demonstrated significantly greater increases in pupillary responses to tropicamide than individuals without the APOE4 allele, although all of the subjects were cognitively normal. Others' findings—that pupillary responses to tropicamide are increased in individuals who have Alzheimer's disease (1), that APOE status alters susceptibility to late-onset Alzheimer's disease, and that individuals with the APOE4 allele are at higher risk for Alzheimer's disease later in life (4, 7)—imply that increased pupillary responses to tropicamide may occur not only in patients with Alzheimer's disease but in some cognitively normal individuals at higher risk for Alzheimer's disease.

Perhaps APOE plays a role in the hypersensitive pupillary responses of patients with Alzheimer's disease in whom the APOE4 allele is overrepresented. Our measurement of pupil dilation responses to tropicamide in relation to APOE genotype in patients with Alzheimer's disease and age-matched control subjects (8) resulted in two observations. 1) Although the mean percent change in pupil size was greater for patients with Alzheimer's disease than for control subjects, there was substantial overlap between the measurements for the two groups, indicating limited utility of this procedure as a diagnostic tool for Alzheimer's disease. 2) Among patients with Alzheimer's disease, those with the APOE4 allele tend to have greater pupil dilation responses than those without the APOE4 allele.

Our findings in normal individuals as well as in patients with Alzheimer's disease led to speculation that APOE is directly or indirectly involved in cholinergic nerve action in the iris. Several lines of evidence have indicated that the role of APOE in the central nervous system is particularly important in relation to the cholinergic system, and that the APOE4 allele has a direct impact on cholinergic dysfunction in the brains of patients with Alzheimer's disease (9). Similar cholinergic dysfunction in the iris may exist in cognitively normal persons with the APOE4 allele and patients with Alzheimer's disease.

These preliminary results suggest that the hypersensitive pupil dilation response to tropicamide is already present in cognitively normal individuals who have the APOE4 allele and thus are considered to be at higher risk for Alzheimer's disease. Moreover, they indicate the possibility that this allele is involved in the mechanism of the hypersensitivity. Further studies examining the relation between other cholinergic functions and APOE status may provide more insight into this issue.

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