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Ageing and Alpha$_1$ Adrenoceptors in the Iris

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Summary
This study was undertaken to determine if the age related decrease in pupil size is attributable to altered sympathetic adrenoceptor responsiveness. Log dose response curves using the selective alpha$_1$ agonist phenylephrine and antagonist thymoxamine showed that there is no difference in alpha$_1$ adrenoceptor sensitivity in the elderly and the young. Therefore age related miosis is not caused by an alteration in alpha$_1$ receptors with age.

Pupil size is controlled by the opposing actions of the parasympathetic and sympathetic nerves. Changes in pupil size provide a useful model for the study of the autonomic nervous system, particularly as selective agonists and antagonists for autonomic receptors are available for topical application in the eye.

In the normal population a decrease in pupil size with age is well established.[1] The precise mechanisms involved remain in doubt and possible explanations include degenerative changes in the muscles of the iris,[1] degeneration and segmental demyelination of autonomic nerve fibres[2] or ganglion cells,[3] parasympathetic escape due to reduction in central inhibition[1] or reduction in sympathetic tone.[4] The present study was undertaken to determine if age related miosis is attributable to altered adrenoceptor responsiveness. Alpha$_1$ adrenoceptors mediate the contraction of the dilator pupillae muscle. Log dose response curves using the selective alpha$_1$ agonist, phenylephrine, and antagonist, thymoxamine, were constructed to determine the sensitivity of alpha$_1$ adrenoceptors in the elderly and the young. The possible effects of ageing on alpha$_1$ adrenoceptor mediated function have been little studied in man and the available data are inconclusive.[5]

Material and Methods

Subjects
Sixty healthy subjects were included in the study. Only those with blue-grey irides were included as the mydriatic response to sympathomimetic amines is inversely proportional to the degree of pigmentation of the iris,[6] and the elimination of green, hazel or brown eyes served to reduce variability in response. Subjects were sub-divided into two groups. Group A were aged between 20 and 35 years (mean age of 23 years) and group B were aged between 65 and 90 years (mean age of 72 years). Each subject had a complete medical history taken and had a full physical examination. Ophthalmological assessment included pupillary reflexes, corrected visual acuity, slit-lamp and fundal examination. Excluded from the study were monocular subjects and those with a corrected visual acuity of less than 6/12, subjects with ocular pathology or history of previous ophthalmic surgery. Also excluded were those with systemic disease and those on medication likely to affect the autonomic nervous system.

Smoking and caffeine-containing beverages were not permitted on the day of the study. Alcohol was prohibited during the twelve-hour period preceding and throughout the study.

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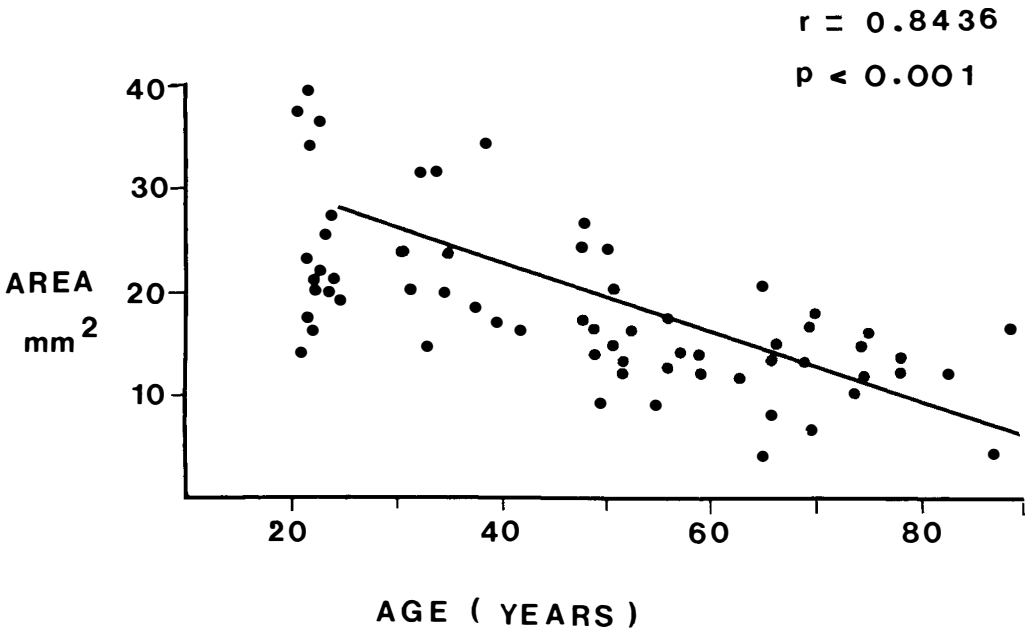

Fig. 1. *Linear regression analysis for 64 subjects showing significant negative correlation between resting pupil area and age ($r = 0.84$, $p<0.001$).*

The protocol was approved by the Ethics Committee of the Royal Victoria Eye and Ear Hospital, Dublin, and accepted by the National Drugs Advisory Board. Written, informed, consent was obtained from all subjects.

Methods

To study the effect of a selective alpha$_1$ agonist, 10 per cent phenylephrine (manufactured by Richard Daniels) was used and diluted with water for injection to concentrations of 0.625 per cent, 1.25 per cent, 2.5 per cent and 5 per cent. A given dose of phenylephrine was administered to eight different subjects, four in group A and four in group B. An estimated volume of 60 microlitres at room temperature was placed with a Pasteur pipette in the conjunctival sac of the eye under study. The other eye acted as a control.

The effect of phenylephrine following the use of a selective alpha$_1$ antagonist was also studied. A commercial preparation of 0.5 per cent thymoxamine (Smith and Nephew Ltd) was diluted with water for injection to 0.25 per cent. Twenty subjects (ten in group A, ten in group B) received 60 microlitres 0.25 per cent thymoxamine in both eyes. Ten per cent phenylephrine was instilled ten minutes after the thymoxamine into the right eye, the left eye acting as a control.

Measurements were recorded using static pupillometry. Subjects were seated at a modified slit-lamp table. Photographs were taken every five minutes for a two hour period with a Nikon FM2 camera using Kodak Plus X 35 mm film. The camera was mounted on a focusing stage at a fixed distance from each subject. Background illumination was diffuse and constant at 88 lux.

Photographic negatives were projected onto a Calcomp 2000 digitising tablet. The tablet was connected to an Apple II Europlus computer with monitor, disc drives and Epson FX80 printer. A commercial computer program, the VIDS general measurement program, was used to measure pupil area. A millimetre scale was attached to the slitlamp headrest and a calibration procedure was incorporated in the VIDS program to ensure comparable measurements between subjects.

Results

Linear regression analysis in 64 subjects confirmed a highly significant negative correlation between resting pupil area and age (Fig. 1). The marked difference in pupil area at the extremes of age determined the selection of subjects under the age of 35 years and over the age of 65 years.

Photographs were taken over a two hour period to establish the time course of action of phenylephrine in the two age groups. Maximal mydriasis was achieved with all con-

Table I *Maximum mydriasis produced by 10 per cent topical phenylephrine in young and elderly subjects expressed both as a percentage change in area of the experimental eye relative to the control, and as an absolute value in mm². Values are mean±SE of mean from 4 subjects in each group*

		Mean maximum mydriasis	
	Age	Per cent	mm²
Group A	<35	98.28±16.75	48.56±3.6
Group B	>65	282.38±20.5	50.89±1.0

centrations of phenylephrine and in both age groups at between 50 and 70 minutes. Measurement of the mydriatic response was taken at one hour after dosage with phenylephrine throughout the study.

Log dose phenylephrine plotted against the mydriatic response expressed as a percentage change showed a significantly greater maximum response in the elderly (Table I). In group A, the mean percentage change in area ranged from 41.23 per cent±4.79 (SEM) with 0.625 per cent phenylephrine to 98.28 per cent±16.75 (SEM) with 10 per cent phenylephrine. In group B, the mean maximum response increased from 146.77 per cent±18.31 (SEM) with 0.625 per cent phenylephrine to 282.38 per cent±20.5 (SEM) with 10 per cent phenylephrine. Log dose phenylephrine plotted against the mydriatic response expressed as an absolute value showed that the same final diameter was achieved in both age groups (Table I and Fig, 2). In group A, the mean pupil area increased from 28.94 mm²±4.4 (SEM) to 48.56 mm²±3.6 (SEM) with concentrations from 0.625 per cent to 10 per cent phenylephrine. In group B, a similar increase in mean pupil area from 27.64 mm²±4.7 (SEM) to 50.89 mm²±1.0 (SEM) was achieved with the same dose range of phenylephrine.

A similar degree of miosis occurred in the elderly and the young in response to topical 0.25 per cent thymoxamine. The mean pupil area decreased from 21.68 mm²±2.3 (SEM) to 11.33 mm²±1.23 (SEM) in group A and from 14.52 mm²±2.04 (SEM) to 9.37 mm²±1.28 (SEM) in group B. The difference in mean area reached in both group A and group B was not statistically significant (Fig. 3).

The mydriatic response to topical 10 per cent phenylephrine instilled ten minutes after 0.25 per cent thymoxamine was similar in both age groups when expressed in absolute values (Fig. 3). The mean pupil area increased to 17 mm²±1.91 (SEM) in group A and 18.17 mm²±2.35 (SEM) in group B. The difference in mean area achieved was not statistically significant.

Discussion

Autonomic disorders and altered drug responsiveness are more common in the elderly,[7] but very little is known about the effect of age on the autonomic nervous sytem. Histological studies of the ciliary and superior cervical ganglia have demonstrated increased degenerative changes with age in both ganglia.[3] Likewise, in the rat heart, catecholamine content decreases with age,[8] but this may be due mainly to axon degeneration, since there is no decrease of noradrenergic vesicles in the remaining nerve terminals.[9] Such a loss of noradrenergic nerves may explain why pupil diameter is reduced in the elderly. In the present study the difference in pupil diameter between young and old was eliminated by thymoxamine, presumably by abolishing sympathetic dilator tone.

In relation to neuro-transmitters it has been shown that plasma noradrenaline levels increase with age in man. This may be due to a reduced clearance from the plasma[10] or due to increased transmitter release.[11]

At the receptor level, it is well established that beta adrenoceptor responsiveness in many preparations is reduced in the elderly.[7]

Human platelets provide a convenient source of alpha$_2$ adrenoceptors, and a recent study has shown no age-related changes in number or affinity of binding sites.[12] In animal studies, alpha adrenoceptor mediated responses seemed to be diminished with age, especially in the rat aorta,[13] but not in the rat vas deferens.[14]

The responses of human isolated arteries and veins to noradrenaline have been studied. The sensitivity to noradrenaline has been shown to be unaltered with increasing age.[15,16] In addition, forearm blood flow shows no age related change with the non-selective alpha antagonist phentolamine.[17] We are aware of

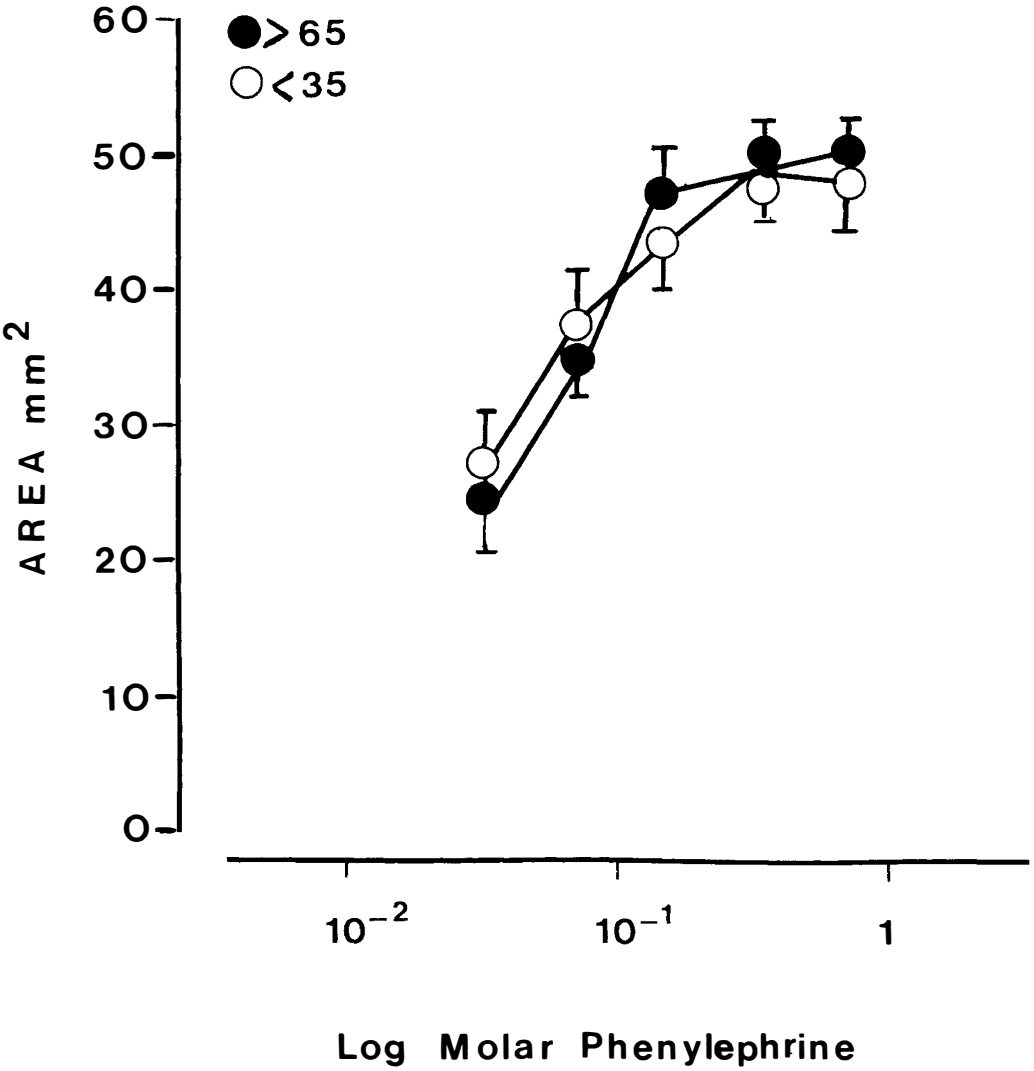

Fig. 2. *Effects of topical 0.625 per cent, 1.25 per cent, 2.5 per cent, 5 per cent and 10 per cent phenylephrine on absolute pupil area (mm^2) in young and elderly subjects. Vertical bars represent S.E. of mean from 4 subjects.*

only one report showing a reduction in pressor potency by the $alpha_1$ selective agonist phenylephrine with increasing age in man.[18]

In the eye, $alpha_1$ adrenoceptors mediate the contraction of the dilator pupillae muscle. In the present study the mydriatic response to the selective $alpha_1$ agonist phenylephrine was considerably greater in the elderly than in the young, when expressed as a percentage change in area, but not when expressed as an absolute value.

To investigate this apparent increased sensitivity to phenylephrine the selective $alpha_1$ antagonist thymoxamine was used and found to abolish the difference in base-line pupil size. Ten per cent phenylephrine administered with 0.25 per cent thymoxamine produced a similar mydriatic response in both young and old. Having abolished the increased sympathetic tone in the young and the resulting difference in baseline area, the sensitivity to phenylephrine of the dilator pupillae was unchanged with age. This demonstrates that apparent differences in

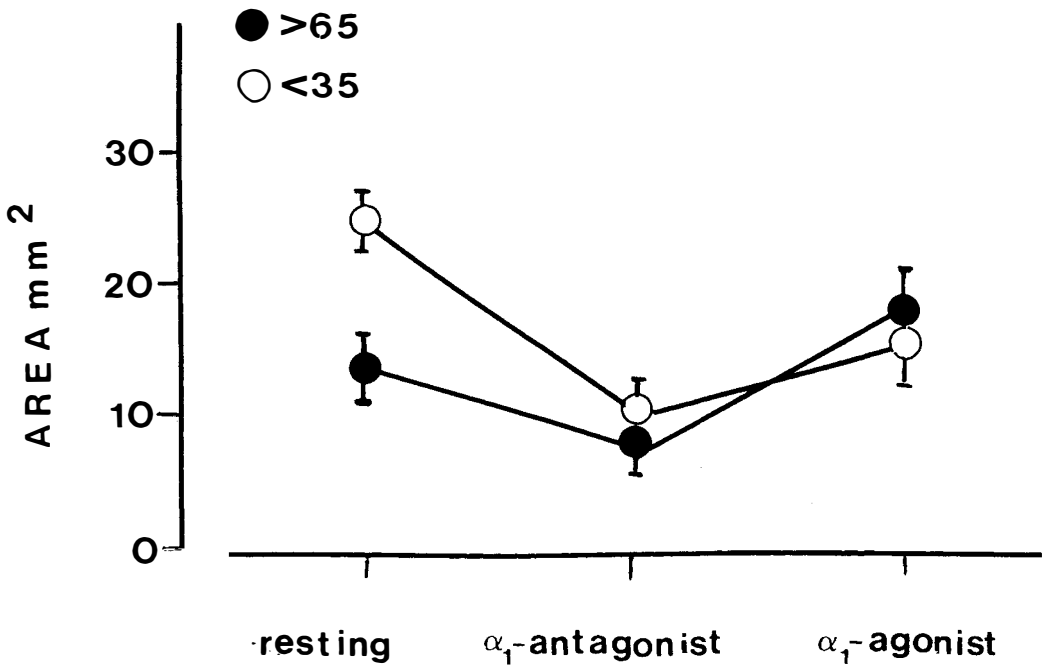

Fig. 3. *Effects of topical 0.25 per cent thymoxamine followed by topical 10 per cent phenylephrine on absolute pupil area (mm^2) in young and elderly subjects. Vertical bars represent S.E. of mean for 10 subjects in each group.*

sensitivity to phenylephrine (in percentage terms) are due to differences in resting pupil diameter.

In conclusion there is no evidence to suggest that the age related decrease in pupil area is caused by an alteration in alpha$_1$ adrenoceptors with age. This finding would agree with studies on vascular alpha$_1$ adrenoceptors where responsiveness is unchanged or decreased in ageing.

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