
Effect of topical carbachol on the pupil and refraction in young and presbyopic monkeys

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*Pupil diameter and refractive state were measured after carbachol was applied onto the cornea or injected into the anterior chamber of cynomolgus monkeys (*Macaca irus*) and vervets (*Cercopithecus aethiops*). The results are presented as dose-response curves. The refraction dose-response curves are expressed in both diopters and per cent of maximal accommodation. No important species differences were observed. ED_{50} for refractive change after carbachol was placed onto the cornea was approximately 30 mcg., and after injection into the anterior chamber it was 0.12 to 0.18 mcg. ED_{50} for pupil contraction after carbachol was applied onto the cornea was 0.3 to 0.5 mcg., and after carbachol was injected into the anterior chamber it was 0.005 to 0.006 mcg. An accommodation amplitude (determined after drug stimulation) of 15 to 20 D. is common among young monkeys. Two of the monkeys in the present series were presbyopic. They had flat dose-response curves, i.e., needed a stronger pharmacologic stimulus per diopter of accommodation.*

Pilocarpine and carbachol are two of the drugs commonly used in the clinical treatment of glaucoma. The effect of pilocarpine on pupil size and on refraction in monkeys has been previously reported.^{1,2} In vitro and in vivo pupil experiments were done by Velhagen¹⁴ when carbachol was introduced into clinical ophthalmology. He also performed studies of refractive changes after single doses of pilocarpine, eserine, and carbachol given topically to the eye. However, these experiments were done on only one person. The clinical value of carbachol as a glau-

coma drug has been reported in more recent papers.⁷

The present paper gives dose-response curves of the effect of carbachol on the pupil and on refraction in living monkeys. The results are compared with those obtained with pilocarpine under the same conditions. Presbyopia in monkeys is also discussed.

Materials and methods

Six cynomolgus monkeys (*Macaca irus*) weighing 1.3 to 2.4 Kg. and six vervets (*Cercopithecus aethiops*) weighing 2.5 to 4.1 Kg. were used. Among the cynomolgus monkeys there were one preadolescent male (1.3 Kg.) and 5 adult females. Two of the females were judged to be aged because of their worn-out teeth and their generally older features. The vervets were all young adults.

Fig. 1 shows the sex and age of the monkeys and gives the corresponding symbol for each. The same symbol has been used to designate each animal in Figs. 3 to 5, 7, and 8.

Since the experimental methods have been de-

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Age group	Cynomolgus		Vervet	
	male	female	male	female
preadolescent	X			
young adult		■ ▲ ●	△ □ ●	■ ● ■
old		■ ■		

Fig. 1. The symbols for the monkeys used in Figs. 3 to 9 and the ages of the monkeys as estimated from the teeth and general body features.

scribed in detail previously,¹² only the essentials will be given here. All animals were prepared by having an iridectomy performed on the right eye, first in the 12 o'clock position and several weeks later in the 6 o'clock position. It has been shown that iridectomy has no influence on the refraction of monkeys.¹² During the experiments the animals were anesthetized with intraperitoneal pentobarbital, 30 mg. per kilogram of body weight.

Pupil experiments. After a monkey was anesthetized, the experiment was begun with the pupil measurements on the left eye. The pupil diameter was measured with a pair of vernier calipers under constant illumination. Four single readings in rapid succession were taken, and the mean calculated. During induction of anesthesia the pupil size varied considerably, but once the barbiturate had taken full effect, only small changes in pupil diameter occurred even during prolonged anesthesia. The carbachol was not given until a steady state was reached after the induction of anesthesia (Fig. 2). After carbachol administration the pupil diameter was followed with sets of four readings, until a definite minimal diameter was obtained.

Refraction experiments. The refraction measurements were performed on the iridectomized eye after the pupil experiments were finished. The refraction was read from a Thorner¹¹ optometer, which gives the spectacle plane refraction in diopters (D.). A prevalue was taken as the mean of 10 single readings, and after the drug was given (Fig. 2) the refraction was followed until a definite maximum was obtained (final value). The values after carbachol administration were taken as the mean of four single readings in rapid succession. Prolonged anesthesia caused no significant change of the refractive state.

Carbachol administration. The carbachol was either applied onto the cornea or injected into the anterior chamber. When carbachol chloride was to be applied onto the cornea, the dose of the drug was given in 10 μ l of distilled water. During 2 minutes the 10 μ l of the drug solution was applied centrally onto the cornea with an Agla micrometer syringe (Burroughs Wellcome, Ltd., London), and the lids were kept open for another

2 minutes. When 20 mg. of carbachol was given onto the cornea for determination of a maximal refraction response, this dose was given in 0.2 ml. of distilled water during a 10 minute interval. When the drug was applied onto the cornea, one or at most two doses were tested during the same period of anesthesia. This was true for experiments on both pupil size and refraction. When carbachol was injected into the anterior chamber, the eye was punctured with a stainless steel needle. This was connected to an Agla micrometer syringe by polyethylene tubing.

For injection into the anterior chamber, each drug dosage was administered in 1 μ l of normal saline.¹² The solutions were arranged in the tubing in the following order: First, 2 μ l (about 1 per cent of the anterior chamber volume) of 0.9 per cent NaCl, then 1 μ l of air, followed by 2 to 4 increasing doses of carbachol, each two of which were also separated by 1 μ l of air. With this arrangement, several predetermined doses could be given during the same puncture. There was minimal dilution of the natural aqueous humor, and the stronger carbachol doses did not contaminate the weaker ones. This experiment was done aseptically.

When more than one dose was given to the same eye during the same period of anesthesia, corneally or intracamerally, the ratio between successive doses was never less than 10:1. As a consequence, the effect of the first and smaller dose could be neglected besides the second, larger one. All doses refer to the chloride of carbachol.

Calculations. Most results are given as dose-response curves. In the pupil experiments, the smallest pupil diameter reached after each dose of carbachol represented the "response" in the curves. This evidently is unconventional, since the prevalue of the pupil was not taken into account and therefore the "response" does not represent a change. This procedure was adopted because including the prevalue into the expression of the response gives more variability than disregarding the prevalue. (However, the prevalues had to be used in the calculations of the ED₅₀ doses; see below). The smallest diameter after

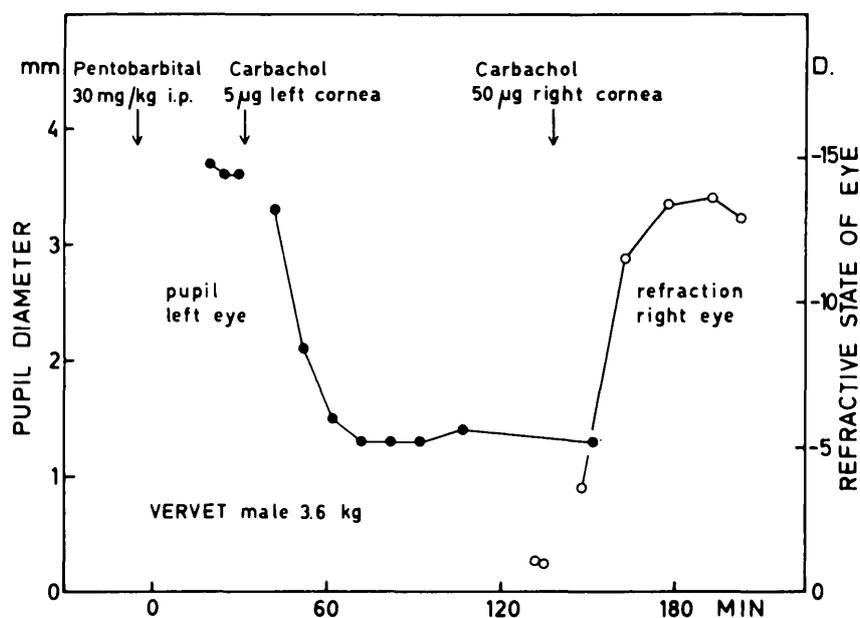


Fig. 2. Pupil diameter (solid circles) of the left eye and refractive state of the right eye (clear circles) in a typical experiment with carbachol applied onto the cornea. The monkey was asleep at time 0. Each circle represents the mean of four single readings, except the clear circle immediately before the 50 mcg. dose, which represents the mean of 10 readings.

each dose was plotted against the logarithm of the dose and the points for each monkey were joined by straight lines. These graphs were used for the calculation of a geometric mean curve for each species and each mode of drug administration as follows: The log dose which caused a certain pupil diameter for each animal and each mode of drug administration was determined from the curves. These log doses were then averaged, and this mean represented the logarithm of the geometric mean dose at the given pupil diameter. The different pupil diameters were plotted against these geometric mean doses, resulting in the geometric mean curves.

To facilitate comparisons, ED_{50} doses for the pupil size were obtained in the following way: The pupil diameter halfway between the average of the prevalues and the average of the minimum diameters (after supramaximal carbachol doses) was calculated for each species, and the corresponding dose read on the geometric mean curve.

In the refraction experiments, the effect was expressed as the largest change in refraction (difference between final value and prevalue). The plotting was done and geometric mean curves were calculated in the same way as in the pupil experiments.

As maximal accommodation responses with supramaximal doses were determined for all monkeys, dose-response curves in terms of per cent

of maximal response could easily be calculated. This was done for all individuals and both modes of administration (although individual curves are shown only for the cynomolgus monkeys; Fig. 5). Geometric mean curves giving the response in per cent of the maximum were calculated for both species in the way described. The ED_{50} doses for refractive change can thus be directly read from these mean curves.

Reliability of the methods. The optometer was tested with trial lenses, and never showed errors of more than ± 0.25 D. The optometer is designed for refraction values up to 20 D. In this investigation, a myopia of more than 20 D. was obtained in a few experiments. This means that some of the values above and just below 20 D. in the figures are only approximate. These values do not influence the mean curves.

The standard deviation of single reading due to error of the method (σ_M) was calculated by means of successive differences. In the pupil experiments σ_M was less than ± 0.15 mm. This was true for both modes of carbachol administration.

In the refraction experiments with corneal administration, σ_M was as follows: readings before carbachol: ± 0.38 D. (233 readings); final refraction readings where a myopia of more than 15 D. was obtained: ± 0.79 D. (70 readings). When carbachol was injected into the anterior chamber, the corresponding values were ± 0.68 D.

(163 readings) and ± 0.82 D. (52 readings). No significant differences between species were found in the standard deviation calculations, and the above figures are combined values for both species.

Astigmatism. The average for prevalues in experiments where no puncture of the cornea had been done was 0.6 D. (both species together). The iridectomies which had preceded the experiments by several months left very little astigmatism.¹² No change in astigmatism was observed during experiments with corneally given carbachol.

In experiments where carbachol was injected into the anterior chamber, the astigmatic conditions often changed with the puncture of the eye. The mean change was 2.9 D. (range 9.3 to 0.5 D.). In all experiments but one, the astigmatism caused by the needle going through the cornea remained unaltered during the experiment. That is, if the difference in optic power between two perpendicular meridians was 5 D. after the eye was punctured but before any carbachol was injected, the same astigmatism was found also after carbachol. In the one experiment where an astigmatic change (2 D.) between pre- and final values was observed, the change in refraction was taken as the mean of the changes in two perpendicular meridians.

Blood pressure measurements. In a few experiments a polyethylene tube was inserted through the brachial artery into the subclavian artery. The blood pressure was followed with a pressure transducer (EMT 456, Elema-Schönander, Stockholm, Sweden) and recorded with a Mingograph (type 24 B, Elema-Schönander).

Results

Changes in refraction after carbachol was applied onto the cornea. The 5 cynomolgus monkeys had a mean refraction prevalue of -0.8 D. (range $+1.7$ to -3.3 D.). The corresponding values for the 5 vervets were -0.7 D. (range $+0.2$ to -1.8 D.). The average time elapsing after carbachol until a full effect was seen was 46 minutes, with a range of 23 to 80 minutes. (Unless otherwise stated, figures given will refer to both species taken together).

The relation between the dose of carbachol placed onto the cornea and the change in refraction is shown in the right-hand groups of curves in Fig. 3 (cynomolgus monkeys) and Fig. 4 (vervets). The dashed curves in the figures are geometric mean curves for the other species of monkey (i.e., the mean curve for vervets is

dashed in the cynomolgus figure and vice versa). Though there is a certain scatter among the individual curves, it is quite obvious that both species of monkey investigated have grossly the same sensitivity to corneally given carbachol.

The maximum obtainable changes in refraction after large corneal doses of carbachol (about 20 mg.) are indicated far to the right in Figs. 3 and 4. These maximal accommodation amplitudes ranged from 9.4 to 21.7 D. for the cynomolgus, and 18.0 to 23 D. for the vervets. The two cynomolgus females which were considered old had the smallest accommodative ability: 9.4 and 9.7 D. These two monkeys also showed the flattest dose-response curves (Fig. 3).

Changes in refraction after carbachol was injected into the anterior chamber.

An injection of 2 μ l of physiologic saline always preceded the carbachol injections. No accommodative response was seen after the saline. Having the eyes cannulated for 1.5 hours caused no significant refractive changes in two monkeys tested. A full effect on refraction was reached in 7 to 18 minutes (mean: 12 minutes) after the carbachol injection.

The effect of different carbachol doses is seen in Figs. 3 and 4 (left hand group of curves). The vervets used here seem slightly more sensitive to intracamerally given carbachol than the cynomolgus monkeys, when the dioptric values (Figs. 3 and 4 or 9) are compared. It is also clear (Figs. 3, 4, or 9) that when carbachol is placed onto the cornea the dose must be 150 to 300 times larger than when it is injected into the anterior chamber to cause the same amount of accommodation. This is true for both species. The two modes of carbachol administration are quite comparable, as four of the individuals of each species used for the corneal application were also used for intracameral injection.

One of the old cynomolgus monkeys with a small accommodation amplitude was used also for the intracameral admin-

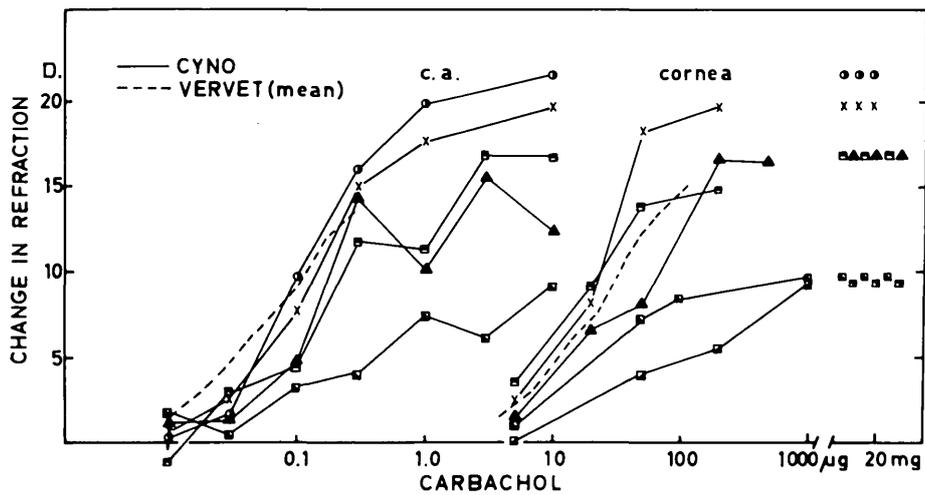


Fig. 3. Refraction effect in the cynomolgus monkey (*Macaca irus*) of carbachol applied onto the cornea and into the anterior chamber. Five cynomolgus monkeys for each mode of administration and mean curves for five vervets (dashes). To the right, the maximally obtainable changes in refraction after large doses of carbachol are indicated (with 3 points each) for the six cynomolgus monkeys. (One monkey is not common to the two modes of administration.)

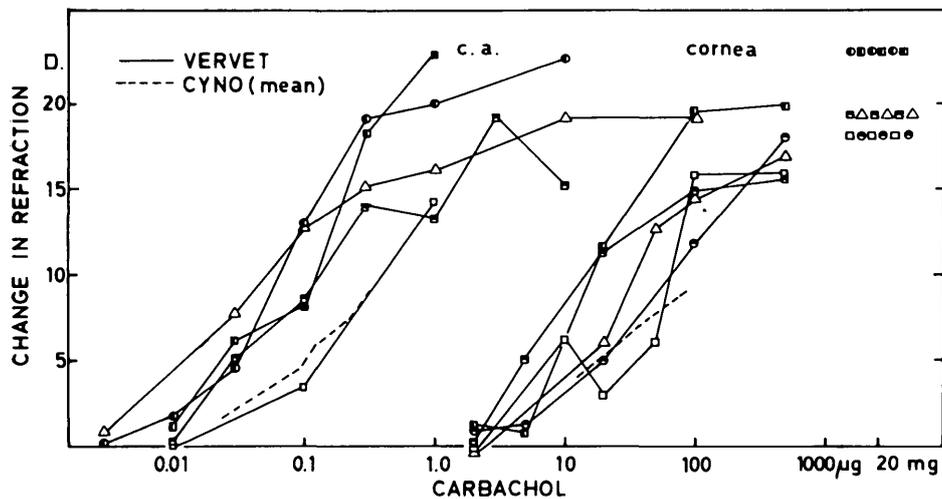


Fig. 4. Refraction effect in the vervet (*Cercopithecus aethiops*) of carbachol administered onto the cornea and into the anterior chamber. Five vervets for each mode of administration and mean curves for five cynomolgus monkeys (dashes). To the right, the maximally obtainable changes in refraction after large doses of carbachol are indicated (with 3 points each) for the six vervets. (One monkey is not common to the two modes of administration.)

istration, and once more showed the flat dose-response curve (Fig. 3).

Because of the wide scatter of the maximal accommodation amplitude among different individuals, dose-response curves were calculated as per cent of maximal accommodation instead of diopters. Fig.

5 shows this type of dose-response curves for the cynomolgus species. It is obvious (by comparing Figs. 3 and 5) that the curves lie closer together using this way of representing the results. Geometric mean curves expressing the results in per cent are given in Fig. 6. From these curves

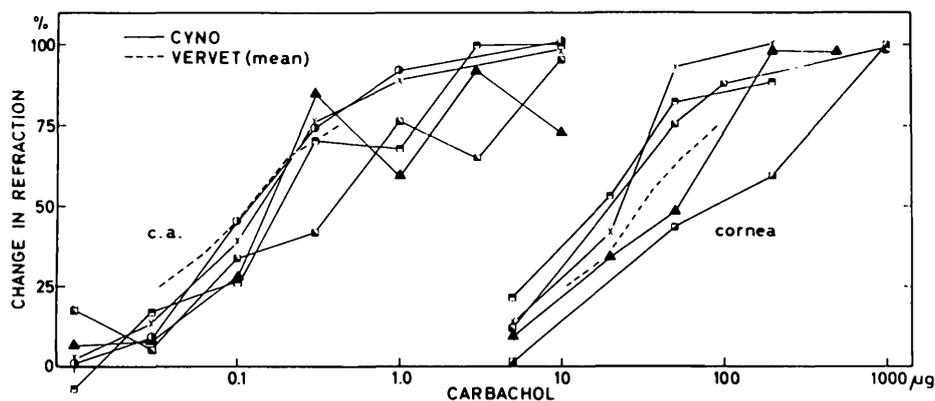


Fig. 5. Refraction effect of carbachol in cynomolgus monkeys (*Macaca irus*), five monkeys for each mode of administration. The responses are expressed in per cent of the accommodation amplitudes of the individual monkeys. The dashed curves are mean curves for vervets.

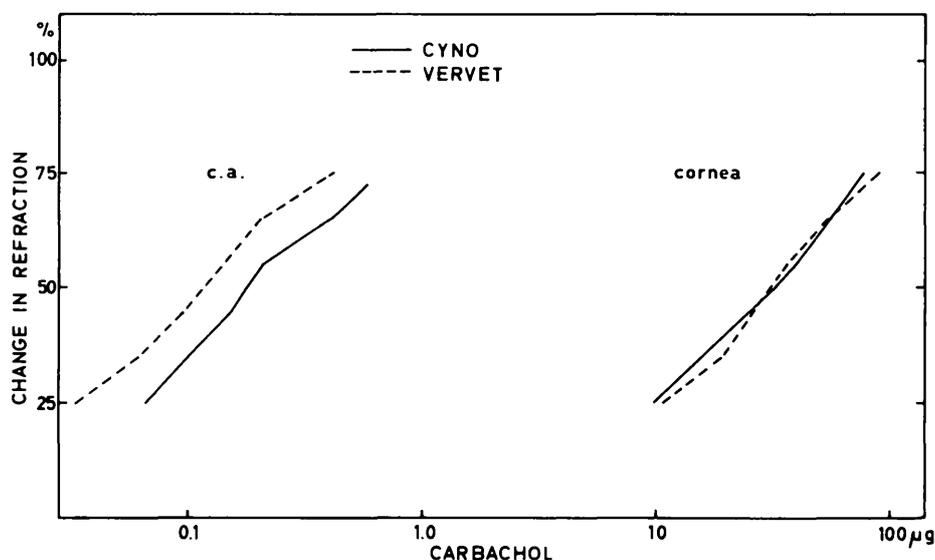


Fig. 6. Geometric mean curves for cynomolgus monkeys (*Macaca irus*) and vervets (*Cercopithecus aethiops*) showing the change in refraction, expressed in per cent of maximum, after carbachol applied onto the cornea and injected into the anterior chamber.

the doses necessary to cause a 50 per cent effect on accommodation (ED_{50}) can be estimated as 30 mcg. of carbachol for the corneal administration and 0.12 to 0.18 mcg. for intracameral injection.

Effect on the pupil of carbachol application onto the cornea. The means of the prevalues of the pupils were 3.0 mm. (range 2.6 to 3.5 mm.) for the cynomolgus monkeys and 3.4 mm. (range 2.7 to 4.0 mm.) for the vervets. Full effect on the

pupils was obtained on an average in 37 minutes (range 15 to 70 minutes) after carbachol was applied. The maximal miosis after large doses of carbachol (2 mg.) was determined for the five monkeys of each species used. The means of the minimum diameters were 1.2 mm. (range 1.0 to 1.4 mm.) for the cynomolgus and 1.3 mm. (range 1.1 to 1.4 mm.) for the vervets. The dose-response curves for the individuals are shown in Figs. 7 and 8.

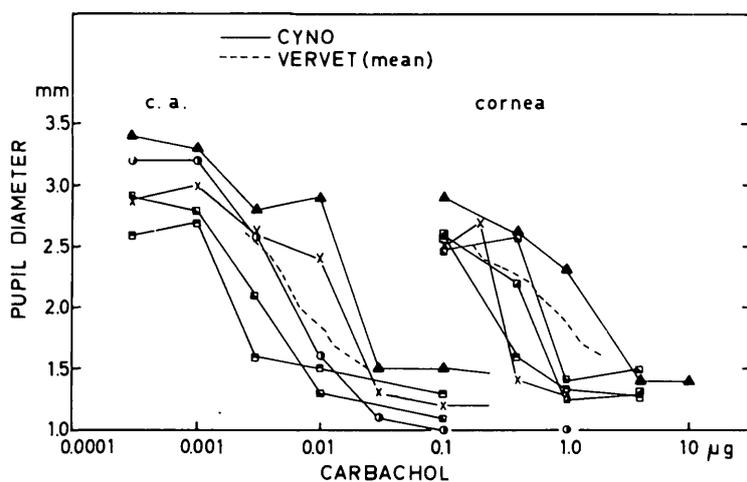


Fig. 7. Pupil diameter after carbachol applied onto the cornea and into the anterior chamber. Five cynomolgus monkeys (*Macaca irus*) and geometric mean curves for five vervets (dashes).

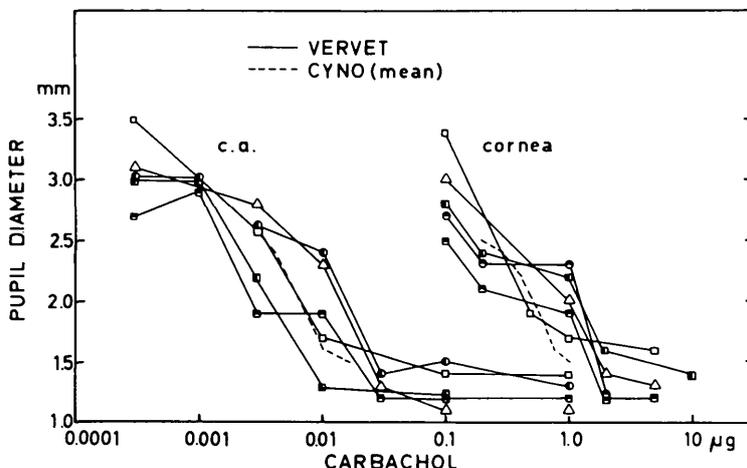


Fig. 8. Pupil diameter after carbachol applied onto the cornea and into the anterior chamber. Five vervets (*Cercopithecus aethiops*) and geometric mean curves for five cynomolgus monkeys (dashes).

A maximal effect on the pupil is obtained with 1 to 5 mcg., whereas doses less than 0.1 mcg. caused little change in the pupil diameter. (The curves do not show these small doses.) Comparison of the mean curves (Fig. 9) seems to show that the cynomolgus monkeys are more sensitive to carbachol given corneally than the vervets, and that they have a steeper dose-response curve.

Half maximal responses of the pupil are obtained (see calculations) when approxi-

mately 0.5 mcg. of carbachol is given to cynomolgus monkeys and 0.3 mcg. to vervets. Thus, with this way of stating the results, the vervets seem more sensitive. The corneal ED_{50} dose for refraction was about 30 mcg. for both species (Fig. 6). Thus ED_{50} for the pupil is 50 to 100 times less than that for refraction with this substance and mode of application.

Effect on the pupil of carbachol injection into the anterior chamber. A 2 μ l amount of saline was always injected into

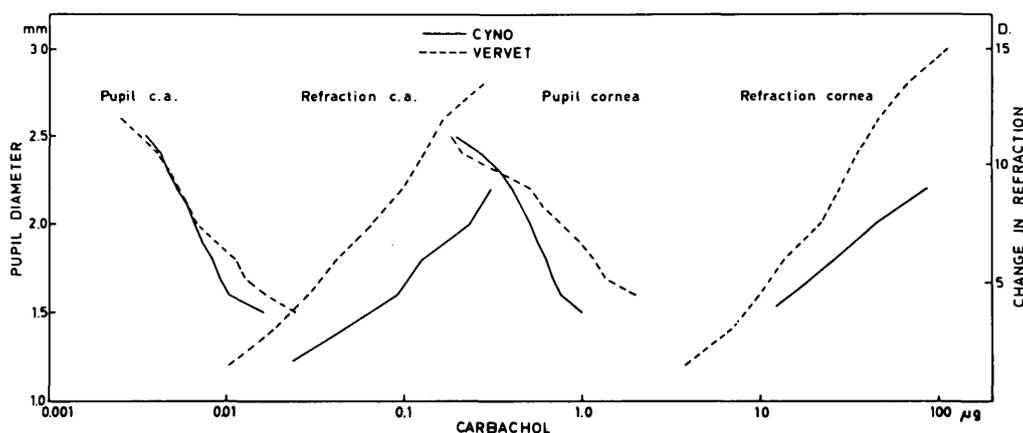


Fig. 9. Geometric mean curves for five cynomolgus monkeys (*Macaca irus*) and five vervets (*Cercopithecus aethiops*) showing the effect on the pupil diameter and on change in refraction (in diopter) of carbachol applied into the anterior chamber and onto the cornea.

the anterior chamber prior to the carbachol doses, but it never caused changes in pupil diameter bigger than 0.1 mm. over 15 minutes. Two injections in one monkey and 3 in another of 2 μ l of 0.9 per cent NaCl (without drug) were performed at 15 minute intervals. The pupil dilated or constricted only 0.1 to 0.2 mm. during these measurements. The means of the prevalues of the pupils after the eye was punctured, but before anything was injected, were 3.1 mm. (range 3.0 to 3.3 mm.) for the cynomolgus monkeys and 3.3 mm. (range 3.2 to 3.7 mm.) for the vervets. Full effect of the carbachol doses was reached within 2 to 10 minutes (mean 7 minutes). The effect on the pupil of intracamerally injected carbachol is recorded in Figs. 7 and 8. The two species show similar sensitivity. The ED_{50} doses are about 0.006 mcg. for cynomolgus and 0.005 mcg. for vervets (using the values for maximal miosis found after corneal administration). This means that a carbachol dose placed onto the cornea (by the method used here) must be 50 to 100 times larger than an intracamerally injected one to cause the same degree of miosis.

The doses necessary to give a 50 per cent response of refraction after carbachol

injection into the anterior chamber were 0.12 and 0.18 mcg. (Fig. 6). These doses are about 20 to 30 times larger than those giving a 50 per cent response of the pupil.

Systemic effects. The blood pressure was recorded in three cynomolgus monkeys which received 0.1, 1.0, and 20 mg. on one cornea. No change in blood pressure follows 0.1 mg. After 1.0 mg., two monkeys showed a slight decrease in blood pressure: from 110/75 to 90/60 and from 90/65 to 80/50 mm. Hg. The blood pressure rose from 95/60 to 110/75 mm. Hg in the third monkey. In this last monkey 100/70 mm. Hg was recorded even after 20 mg., whereas the other two had blood pressures of 90/65 and 65/40 mm. Hg after this dose.

Salivation occurred after 0.1 to 1.0 mg. onto the cornea. After 20 mg., urination and defecation were sometimes observed. The carbachol doses injected into the anterior chamber caused no positive systemic signs.

Discussion

The investigations of the blood pressures after carbachol applied onto the cornea were carried out because it seems probable, from experiments to be reported later,¹³ that the accommodative mechanism

cannot function well when the blood pressure is very low. Decreases in salivation and blood pressure are two sensitive and easily recordable signs of systemic carbachol action. As only small or no such effects were seen after the carbachol doses used for the dose-response curves in the present paper, the possibility is remote that the local response to carbachol is influenced by systemic actions. Also, after the large doses used for determining the maximal accommodation amplitude, the blood pressure response in two out of three monkeys was small. This can be explained by the loss of a part of the dose through lacrimation and salivation (here perhaps a local more than a systemic effect).

The refraction curves in Fig. 9 seem to show that the cynomolgus monkeys were less sensitive to carbachol doses, whether the drug was administered onto the cornea or into the anterior chamber. That this result is due to the presence of two old, presbyopic monkeys in the cynomolgus group seems probable after the mean curves giving the response in per cent (Fig. 6) are compared. This figure shows that practically no difference in sensitivity can be found between species.

The mean curves (Fig. 9) give the impression that for the corneal route of administration the pupils of the vervets are less sensitive than those of cynomolgus monkeys, but when the ED_{50} doses are compared the vervets are slightly more sensitive. This contradiction is explained by the ability of the cynomolgus monkeys to respond with a higher degree of maximal miosis than the vervets, and by the fact that the vervets on an average had wider pupils initially. Thus, the mean curves are not parallel and the difference between the two species cannot be expressed in a single figure.

Comparative studies on the effects of carbachol on pupil size and on refraction have not been performed before. Without giving dose-response curves, Velhagen¹⁴ found that carbachol could in vitro contract sphincter pupillae of cat and cow in

a concentration of 2.5×10^{-12} Gm. per milliliter. In the present experiments 3×10^{-10} Gm. into the anterior chamber (0.25 ml.) never caused a pupillary contraction but 10^{-9} Gm. sometimes caused a slight contraction. This latter dose gives a concentration about 1000 times greater than that used by Velhagen. That Velhagen found a much greater sensitivity can perhaps be explained by species differences and/or by the fact that giving a drug to an organ with intact blood circulation and suspended in circulating aqueous humor is quite different from the in vitro investigations. Velhagen¹⁴ also found that 2 per cent pilocarpine dropped into the conjunctival sac of rabbits had a much weaker miotic effect than 0.5 per cent carbachol. Comparing the carbachol results in this paper with those obtained with pilocarpine¹² under similar conditions, it is found that if the drugs are given onto the cornea, pilocarpine doses must be 5 to 10 times larger to give the same degree of miosis. If the drugs are injected into the anterior chamber, carbachol is 50 to 100 times more active. The same comparison for the changes in refraction gives similar results. Carbachol is proportionately a far more active substance, compared to pilocarpine, when it is injected into the anterior chamber, than when it is dropped onto the cornea. This can easily be explained by the poorer permeability of carbachol.⁸ The pupil reacted to smaller doses of carbachol than did the accommodation. As has been discussed in an earlier paper,¹² this is probably due to differences in diffusion pathways to the target cells.

Little is known about the maximal accommodative amplitude in monkeys. Hess and Heine,⁴ using retinoscopy found a change of 10 to 12 D. after eserine was dropped into the eye. Electric stimulation of the ciliary body of rhesus monkeys gave a similar response. Also Beer² found an accommodation capacity of 10 D. in three rhesus monkeys. Unfortunately he does not describe the method used. Kahmann⁵ stimulated enucleated eyes and found an ac-

accommodation amplitude of 10.5 to 11 D. in rhesus monkeys. Barrett¹ had two monkeys (species not reported) look at close objects, and using retinoscopy found that the monkeys had a voluntary accommodation of 4 to 5 D. In the present investigation, maximal accommodation amplitudes have been determined with large doses of carbachol for all monkeys used. The values are in agreement with those found after large doses of pilocarpine¹² onto the cornea and exceed the accommodation amplitudes found by earlier investigators. Several of these authors used rhesus macaques. That these monkeys should have a smaller accommodation amplitude than *Cercopithecus aethiops* and *Macaca irus* is not probable. Two adult rhesus monkeys have been tested,¹³ and after corneally given pilocarpine found to have a maximal accommodation of 16 to 17 D. It is more probable that former investigators used old monkeys (though Barrett states that he used young animals) or have used submaximal stimuli. It is unknown whether a maximal voluntary effort to accommodate, as used by Barrett,¹ can produce the same amount of accommodation as stimulation by drugs. In fact, Fincham³ has found that in humans under eserine the pharmacologically induced accommodation is increased beyond the physiologic maximum.

Though it has been assumed that presbyopia develops in monkeys,⁹ figures supporting this statement have never been published. That presbyopia is a reality in monkeys is, however, quite clear from the present paper. That the two monkeys with the three flat dose-response curves (Fig. 3) and the small accommodation amplitudes were old is beyond doubt.

The three presbyopia curves (one old monkey was given carbachol both onto the cornea and into the anterior chamber) in the present investigation show that the older monkeys needed a stronger pharmacologic stimulus per diopter accommodation than the younger animals. One explanation for this could be that the amount

of ciliary muscle shortening which produces a refractive change of one diopter increases with age, e.g., due to changes in the lens. Another explanation could be that the ciliary muscle in old monkeys responds with less contraction to equal amounts of carbachol than a young one, e.g., due to age changes in the muscle itself.^{6, 10} It is quite reasonable to assume that several aging factors (in the lens, the zonule, and the ciliary muscle) influence the shape of the dose-response curves.

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