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A comparison of the pupilloconstrictor effect of pilocarpine solution administered to the conjunctival sac as a single drop or as a continuous infusion in normal subjects

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The usual method of administering drugs to the eye is by instillation of drops of solution into the conjunctival sac. For single-dose treatment and in cases in which short duration of effect is required this time-honoured practice is simple and convenient. When the effect of the drug is required for many hours or when the severity of the condition requires a rapid and efficient onset of effect, repeated drop instillation, sometimes at very frequent intervals, is often used. Of the alternatives to frequent repetition of single-drop instillation for the rapid onset and sustained action of a drug, the two that have received most attention in the past are continuous infusion of a drug solution and sustained release of drug from a contact lens or from a lamella placed on the surface of the eye.

Methods of perfusion of the surface of the eye have ranged from surgical insertion of plastic tubes into the lower fornix (Lippas, 1964) to the use of hard contact lenses with holes bored eccentrically through which fluids have been passed (Morgan, 1971). The volume of fluid delivered in these systems has usually been relatively large and has resulted in a continuous flow of fluid running out of the conjunctival sac with obvious inconvenience and discomfort to the patient. In one method the excess fluid was collected in a cup attached to the patient's cheek (Tan, 1970).

We have devised a method for continuous infusion of a drug solution into the conjunctival sac at a rate which does not cause overflow, which is simple to operate and causes no discomfort. In this paper we describe our use of the measurement of the pupilloconstrictor effect of pilocarpine solutions in normal subjects to compare single-drop administration with continuous infusion. Consideration of the factors which might affect the absorption of pilocarpine also led to an investigation of the influence of the pH of the solution on the rate of onset and the degree of pupilloconstriction.

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Methods

The subjects were healthy volunteers with no history of eye disease, except mild refractive errors, who gave their informed consent to the procedures. Attendances at the laboratory were separated by periods of not less than 24 hours.

MEASUREMENT OF PUPIL DIAMETER

Pupil diameter of one eye was measured with the aid of a low-power binocular dissecting microscope. The microscope, fitted with $\times 5$ eyepieces and a $\times 1{\cdot}25$ objective, was suspended on an extension arm above one eye of the supine subject. All measurements were made in a dark room and a red light source illuminated the eye obliquely at an intensity just sufficient to allow the pupil and iris to be seen through the microscope. Under these circumstances, the pupil was 5 to 8 mm in diameter. One eyepiece of the microscope was fitted with a graticule to allow direct measurement of the diameter of the pupil in the field of view. Measurements were made at five-minute intervals.

ADMINISTRATION OF PILOCARPINE SOLUTIONS

Sterile pilocarpine hydrochloride solution was administered to the eye under observation by placing a single drop into the lower fornix or by constant infusion.

A method was devised for delivering small quantities of fluid continuously into the conjunctival sac without overflow. The delivery system consisted of a length (about 2 m) of silicone elastomer tubing* pushed at one end on to a syringe needle, the sharp point of which had been removed. The tubing (external diameter 0·64 mm, internal diameter 0·31 mm) was chosen because of its softness and pliability (Dohlman, Doane, and Reshmi, 1971). Each needle and tube set was packed individually and autoclaved. Immediately before an infusion, the sterile pilocarpine solution was drawn into a 1 ml disposable tuberculin syringe, the syringe was clamped to a motor-driven infusion ram, the needle carrying the tubing pushed on to the syringe, and the dead space of the tubing filled by starting the motor. The distal end of the tubing was placed in the inner canthus of the eye and held in place by adhesive tape† attached to the

*'Silastic' tubing no. 602–101, Dow-Corning

†'Blenderm' surgical tape, 3M, no. 1525

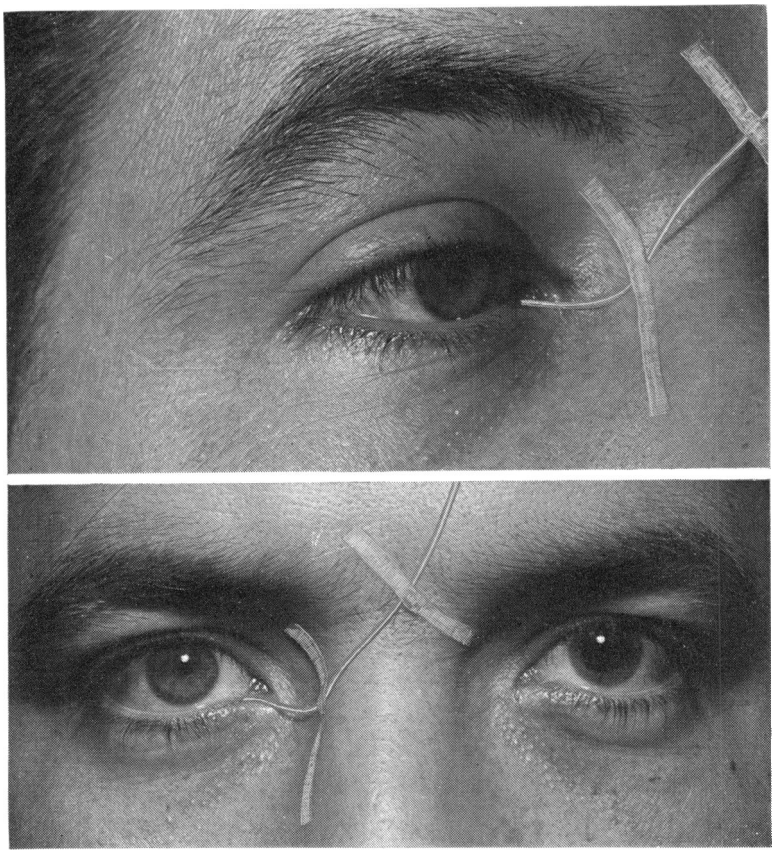

FIG. 1 (a) *The end of the Silastic tubing placed in the inner canthus and held in place by strips of adhesive tape*

FIG. 1 (b) *Flash photograph taken in the darkroom showing the dilated, untreated left pupil and the right pupil constricted by infusion of* 0·01 *per cent pilocarpine solution*

nose and the forehead (Fig. 1). This position was found to be acceptable to all the subjects in that it was comfortable and stable for long periods. The syringe was compressed by the infusion ram at a rate which delivered fluid to the eye at 0·01 ml/min. This rate was selected after measurements with normal saline infusion had shown that this was the maximum rate that could be delivered to the eye without causing overflow in any of the subjects to be used for pilocarpine infusion. The 0·01 per cent pilocarpine solution was used for the infusion experiments so the dose rate was 1 μg/min.

PILOCARPINE SOLUTIONS

The pilocarpine solutions used were of two types.

The first set of solutions came from the hospital pharmacy's routine stock of 1·0 per cent, 0·5 per cent, and 0·25 per cent pilocarpine and the pH was 4·30, 4·70, and 4·95, respectively.

The second set of solutions of 0·5 per cent and 0·01 per cent pilocarpine was specially formulated by the hospital pharmacy and standardized to a pH of 7·2 by the addition of borate buffer. Pilocarpine is much less stable at near neutral pH than at acid pH (Cowle and Anderson, 1967; Riegelman and Vaughan, 1958) so the solution was provided in two bottles, the contents of which were mixed immediately before use. One bottle contained the pilocarpine solution at its natural pH at twice the final concentration required, the other bottle contained an equal volume of an appropriate borate buffer and sodium chloride solution so that on mixing the two solutions, a solution isotonic with tears, having a pH of 7·2 and pilocarpine concentration of 0·5 per cent or 0·01 per cent was obtained.

EXPERIMENTAL PROCEDURE

Measurements of the pupil diameter of one eye were made at five-minute intervals until the diameter had reached a stable baseline. A drop of pilocarpine solution (0·05 ml or 0·025 ml) was then instilled into the lower fornix or the continuous infusion was started (the tubing having been positioned in the inner canthus before measurements began). Measurements were continued at five-minute intervals until the pupil diameter was apparently no longer decreasing or had started to increase again.

Results

RELATIONSHIP BETWEEN PILOCARPINE CONCENTRATION AND MIOTIC EFFECT AT ACID PH

Five subjects each made three separate visits to the laboratory. Measurements of pupil diameter were made for between 75 and 100 min after

instillation of one drop (0·05 ml) of 0·25 per cent (pH 4·95), 0·5 per cent (pH 4·70), or 1·0 per cent (pH 4·30) pilocarpine solution. The effect of each concentration was measured in each subject. For each five-minute interval after drug instillation the change in pupil diameter was expressed as a percentage of the mean of the four baseline measurements made immediately before drug instillation. Fig. 2 shows the means of the percentage reductions at each five-minute interval for the group of five subjects for each concentration of pilocarpine. There was an increase in miotic effect with increase in pilocarpine concentration. The maximum reduction achieved ranged from about 40 per cent for 0·25 per cent pilocarpine to about 60 per cent for 1·0 per cent pilocarpine. The time taken to reach maximum effect was slowest for 0·25 per cent and fastest for 1·0 per cent pilocarpine.

COMPARISON OF THE MIOTIC EFFECT OF 0·5 PER CENT PILOCARPINE AT ACID PH AND AT NEAR NEUTRAL PH

Three subjects each made two separate visits to the laboratory. Measurements of pupil diameter were made for between 65 and 75 minutes after instillation of one drop (0·05 ml) of 0·5 per cent pilocarpine at pH 4·70 or pH 7·2. The effect of pilocarpine at each pH was measured in each subject. Fig. 3 shows the means of the percentage reductions in pupil diameter at each five-minute interval for the group of three subjects for either pH. For 0·5 per cent pilocarpine at pH 4·7 the maximum reduction in pupil diameter and the rate at which it was achieved were almost identical to those shown in Fig. 2, but at pH 7·2 the effect was potentiated, the maximum reduction was about 70 per cent at pH 7·2 compared with about 50 per cent at pH 4·7 and the time taken to reach the maximum was shorter at pH 7·2.

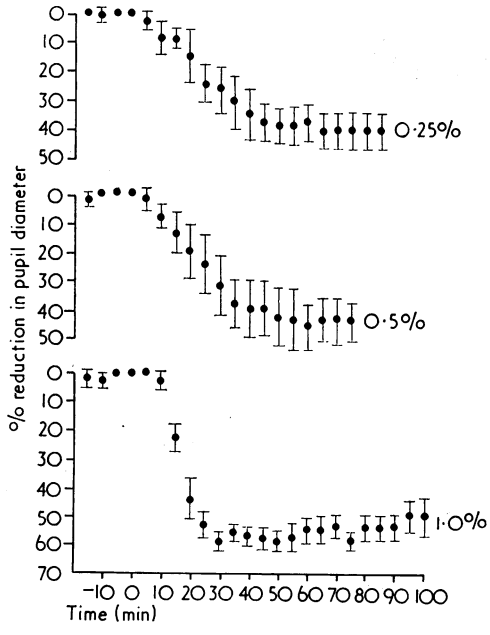

FIG. 2 *The means (±SE) of percentage reduction in pupil diameter produced by single drops of 0·25 per cent, 0·5 per cent, and 1·0 per cent pilocarpine in five subjects. The pilocarpine drop was administered immediately after zero time measurement. Ordinates: reduction in pupil diameter as a percentage of mean of four readings preceding drop administration. Abscissa: time in minutes*

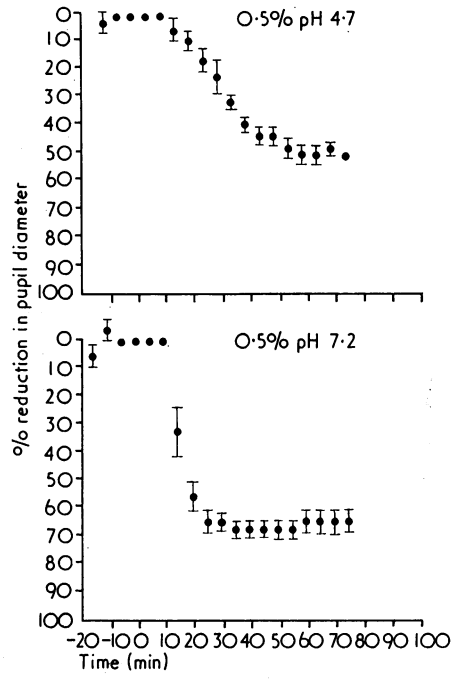

FIG. 3 *The means (±SE) of percentage reduction in pupil diameter produced by single drops of 0·5 per cent pilocarpine at pH 4·7 or pH 7·2 in three subjects. The pilocarpine drop was administered immediately after zero time measurement. Ordinates: reduction in pupil diameter as a percentage of mean of four readings preceding drop administration. Abscissa: time in minutes*

COMPARISON AT PH 7·2 OF THE MIOTIC EFFECT OF PILOCARPINE GIVEN AS A SINGLE DROP OR AS AN INFUSION

Five subjects each made two visits to the laboratory. Measurements of pupil diameter were made for between 75 and 100 minutes after instillation of a single drop (0·025 ml measured by micropipette) of 0·5 per cent pilocarpine (125 μg) at pH 7·2 or

during a continuous infusion of 0·01 per cent pilocarpine (pH 7·2) at the rate of 0·01 ml/min (1 μg/min). The maximum reduction in pupil diameter produced by either procedure was about the same at approximately 60 per cent, but the times taken to achieve this maximum were different; the single drop produced its maximum effect by 30 minutes after instillation whereas the continuous infusion achieved a maximum effect at about 60 minutes after the start of the infusion (Fig. 4).

Discussion

It is generally believed that drugs applied to the conjunctival sac enter the eye mainly through the cornea (Harris, 1968). The successful passage of a drug through a series of biological membranes such as the cornea depends in part on the properties of water solubility and lipid solubility of the drug molecule. For an organic electrolyte such as pilocarpine the pH of the solution will affect the degree of ionization of the molecule and the property

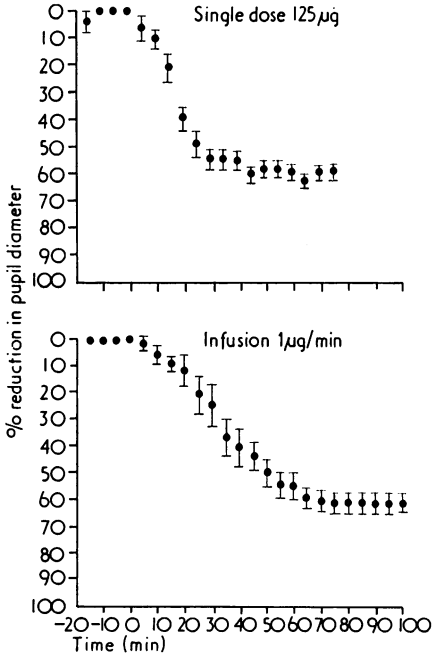

FIG. 4 *The means (±SE) of percentage reduction in pupil diameter produced by a single drop containing 125μg pilocarpine at pH 7·2 or by a continuous infusion of pilocarpine 1 μg/min at pH 7·2 in five subjects. The drop was administered or infusion started immediately after zero time measurements. Ordinates: reduction in pupil diameter as a percentage of mean of four measurements preceding pilocarpine administration. Abscissa: time in minutes*

of water solubility resides in the ionized state, whereas the property of lipid solubility resides in the unionized state (Swan and White, 1942). The ionization constant (pKa) of pilocarpine is 6·87 so that a solution of pilocarpine at pH 6·87 will comprise 50 per cent dissociated (ionized) molecules and 50 per cent undissociated (unionized) molecules. Since pilocarpine is a weak base, lowering the pH by one unit will increase dissociation to 90 per cent while raising the pH by one unit will decrease dissociation to 10 per cent. Thus at the natural pH of hospital stock solutions (0·25 per cent pH 4·95, 0·5 per cent pH 4·70, and 1·0 per cent pH 4·30) pilocarpine will be more than 90 per cent dissociated. On the other hand the proportion of undissociated lipid soluble molecules will be much greater at the near neutral pH of tear fluid. When pilocarpine solution is applied to the eye at its natural pH the degree of absorption will be influenced by a diluting effect of an increase in lacrimation and the ability of the tear fluid to buffer the acid solution towards neutrality. The efficiency of this buffering effect will depend on the pH of the applied solution and on the buffer capacity of the drug used (Cowle and Anderson, 1967). Thus to the variable of concentration of applied drug are added the variables of pH and the ability of the tears to buffer the pH to near neutrality. The relationship between concentration of applied pilocarpine and its effective passage through the cornea as evidenced by its pupilloconstrictor effect would therefore not be expected to be simple. Our results for single doses of 0·25 per cent, 0·5 per cent, and 1·0 per cent pilocarpine showed an increase in effect with increase in concentration but the logarithm of the concentration was not linearly related to the effect. The importance of pH as a factor influencing efficiency of absorption was emphasized in our comparison of the effect of a single drop of 0·5 per cent pilocarpine at pH 4·7 with that at pH 7·2. At near neutral pH the rate of onset and the degree of pupilloconstrictor effect were much greater than at acid pH.

It was on the basis of these observations that it was established that for a meaningful comparison of the effects of a single-drop instillation of pilocarpine with that of a continuous infusion the two solutions should be at the same pH and that the greatest efficiency of absorption from either method would be likely at near neutral pH. The practical disadvantage of using solutions of pilocarpine at neutral pH is the much shorter shelf-life of neutral solutions of pilocarpine compared with acid solutions. In solution, pilocarpine undergoes spontaneous isomerization to isopilocarpine (Riegelman and Vaughan, 1958) which is virtually inactive as a pupilloconstrictor (Anderson and Cowle, 1968). At pH 7·0 about 10 per cent of a

solution of pilocarpine has decomposed in this way at the end of three weeks' storage at room temperature (Cowle and Anderson, 1967; Riegelman and Vaughan, 1958). We overcame this difficulty by preparing separate solutions of drug and of buffer which were mixed immediately before use.

The efficiency with which a low concentration of pilocarpine at neutral pH is absorbed when applied as a continuous infusion was demonstrated in our comparison of the effect of a 0·01 per cent solution given at 0·01 ml/min with that of a 0·5 per cent solution given as a single drop. The single drop of 0·025 ml contained 125 μg pilocarpine but the same degree of pupil constriction had been achieved by the infusion, albeit at a slower rate, at a time when a dose of only 65 or 70 μg had been given. Thereafter the constriction seemed to have reached a plateau of 60 per cent reduction in pupil diameter and presumably would have remained there for as long as the infusion was continued.

When there is a need to produce miosis rapidly, as in the early treatment of acute glaucoma, it is often the practice to instill drops of relatively high concentrations of pilocarpine at frequent intervals in the belief that this is the best way to achieve a rapid effect. Since the pilocarpine drops are strongly acid in high concentration this frequent instillation will counteract the buffering ability of tear fluid and so produce adverse conditions for pilocarpine absorption (Anderson and Cowle, 1968). The repeated administration of eye drops in the early hours of the treatment of acute glaucoma involves frequent disturbance of the patient and is expensive in nursing time. Although conditions in the inflamed eye are different from those used in our investigation it would seem to be logical to administer the pilocarpine at neutral pH and our results suggest that a rapid and sustained miosis will be achieved by the instillation of a single drop of pilocarpine at neutral pH followed by the maintenance of a continuous infusion, at neutral pH, of a much lower concentration of pilocarpine. In this way the amount of disturbance to the patient will be reduced, nursing time will be used more economically, and the total amount of pilocarpine given will be greatly reduced thus decreasing the possibility of systemic toxicity (Epstein and Kaufman, 1965; Greco and Kelman, 1973).

We conclude from our investigation that it is possible to administer a drug by a continuous infusion of a solution into the conjunctival sac at a rate that does not produce tear overflow. The method we have described is simple to use and comfortable for long periods; it is clearly applicable to drugs other than pilocarpine.

Summary

Pilocarpine was administered into the conjunctival sac of normal volunteers by single-drop administration or by continuous infusion of a solution to the inner canthus by means of a fine Silastic tube.

Using pupilloconstriction as a measure of response it was shown that infusion with a 0·01 per cent solution of pilocarpine was as effective as a single drop of 0·5 per cent pilocarpine. The response to the single drop was faster at onset. It was demonstrated that at pH 7·2 pilocarpine was more effective than at acid pH.

The infusion method is simple to use, comfortable for long periods, has potential for reducing the need for frequent drop administration and for reducing the total amount of drug administered, and could be used for drugs other than pilocarpine.

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