DOSE-RESPONSE RELATIONSHIPS IN TROPICAMIDE- INDUCED MYDRIASIS AND CYCLOPLEGIA

S.E. SMITH

Department of Pharmacology, St Thomas's Hospital Medical School, London S.E.1

- 1 Mydriatic and cycloplegic effects of tropicamide in the normal and in the guanethidine-treated human eye have been recorded following prolonged exposure of the eye to drug solutions.
- 2 Marked effects and satisfactory dose-response relationships were observed at drug concentrations within the range 1.25-40 $\mu\text{g/ml}$, by comparison with concentrations of 5-10 mg/ml usually applied as eye-drops.
- 3 The findings are discussed in relation to drug concentrations which produce cholinergic antagonism *in vitro*.

Introduction

Mydriatic agents of the anticholinergic type are conventionally instilled dropwise into the conjunctival sac in concentrations within the range 0.5-1% (5-10 mg/ml). Such concentrations are several orders of magnitude greater than those required for cholinergic antagonism of smooth muscle *in vitro* and evidence suggests that in this respect the iris muscle does not differ greatly from other smooth muscle (Barlow, Franks & Pearson, 1972). The discrepancies in concentrations required for *in vitro* and *in vivo* effects arise presumably because the drugs penetrate poorly through the corneal and scleral cell membranes. It is relevant that atropine, homatropine and cyclopentolate, that are applied in this way, are bases of pK_a values 9.8, 9.9 and 8.4 respectively, which at physiological pH exist largely in the ionized state. It is noteworthy that tropicamide (pK_a 5.37), which is only 2.3% ionized at physiological pH, has effects *in vivo* which are much more rapid in onset and offset than those of the above drugs (Gettes, 1961; Mattila, Takki & Idänpään-Heikkilä, 1967; Smith, 1971), presumably because it is relatively more diffusible through the lipid membranes concerned.

Use has been made here of the ready diffusibility of tropicamide to obtain indirect estimates of drug concentrations in the region of the iris and ciliary muscles necessary to induce mydriasis and cycloplegia *in vivo*. The method used, which involves prolonged drug application, yields satisfactory dose-response relationships. The study has been extended by repetition of the experiments following pretreatment of the eye with

guanethidine, so as to define the influence of the sympathetic nervous system in tropicamide-induced mydriasis.

Methods

All experiments were performed on the left eye of one healthy male subject aged 43 years. Solutions of tropicamide (Mydriacyl-Alcon) in physiological saline were adjusted to pH 7.0 with NaOH, warmed to 34°C and applied to the eye in an eye bath. Application was continued for 100 min with 2 min interruptions at 20 min intervals for measurements of pupil diameter and near-point of accommodation and replacement with fresh solution. Before, during and after drug application pupil diameters were measured photographically in duplicate (Sneddon & Turner, 1967) and near-points of accommodation with a sliding rule in quadruplicate (Smith, 1971). Ambient lighting was artificial only and gave a constant reading of 4.8 on a Weston 'Master' meter.

In one series of experiments, the eye was pretreated with 5% guanethidine sulphate eye-drops (Ismelin-Ciba) twice daily for six days before the series began. At this stage the mydriatic response to the indirect-acting sympathomimetic amine hydroxyamphetamine hydrobromide (1%) (Paredrine-Smith Kline & French) was completely abolished.

The concentration of tropicamide producing 50% mydriasis (EC_{50}) was derived by calculating

Fig. 1 Time course of changes in pupil diameter following prolonged application to the eye of physiological saline ($\square$) and tropicamide 5 $\mu\text{g/ml}$ ($\circ$) in duplicate experiments, and tropicamide 40 $\mu\text{g/ml}$ ($\bullet$) once.

mydriasis at each concentration as a percentage of the maximum obtained independently from repeated dropwise application of cyclopentolate hydrochloride, 10 mg/ml (W.B. Pharmaceuticals). The maximum diameter was 7.75 mm in the normal and 7.05 mm in the guanethidine-pretreated eye. The EC_{50} value for cycloplegia was derived from the decrements in accommodation power (dioptries) expressed as a percentage of the theoretical maximum.

Results

The time courses of changes in pupil diameter and accommodation resulting from application of physiological saline and tropicamide at two concentrations are illustrated in Figures 1 and 2. In each case tropicamide-induced mydriasis and cycloplegia reached maxima at about 80–100 minutes. The 100 min data from these and other experiments have been plotted as equilibrium drug effects against tropicamide concentrations and are shown in Figs. 3 and 4, each of the 13 points being derived from a separate experiment. Tropicamide-induced 50% mydriasis occurred at an approximate concentration of 6 $\mu\text{g/ml}$ ($2.1 \times 10^{-5} \text{ M}$) and 50% cycloplegia at an approximate concentration of 25 $\mu\text{g/ml}$ ($8.8 \times 10^{-5} \text{ M}$).

Drug effects resulting from applications of tropicamide to the eye previously treated with guanethidine are illustrated also in Figures 3 and

Fig. 2 Time course of changes in visual accommodation power following prolonged application to the eye of physiological saline ($\square$) and tropicamide 10 $\mu\text{g/ml}$ ($\circ$) in duplicate experiments and tropicamide 40 $\mu\text{g/ml}$ ($\bullet$) once.

4. The slope of the curve relating mydriatic effect to drug concentration is somewhat steeper than that for the untreated eye, but the drug concentrations inducing 50% mydriasis (7.6 $\mu\text{g/ml}$; $2.7 \times 10^{-5} \text{ M}$) and 50% cycloplegia (28 $\mu\text{g/ml}$; $9.8 \times 10^{-5} \text{ M}$) are of similar magnitude.

In both series of experiments the application of physiological saline induced slight cycloplegia, possibly due to corneal oedema, without change in pupil diameter.

Discussion

The experiments described here indicate that tropicamide, applied to the eye for a long period, dilates the pupil and reduces accommodation for near vision at concentrations which are approximately 1,000 times smaller than those which are usually applied dropwise in clinical practice. This suggests that with traditional instillation of drops into the conjunctival sac only a small proportion of the drug actually penetrates into the eye, even with a compound as freely diffusible as tropicamide. With less diffusible compounds such as cyclopentolate and homatropine it seems likely that an even smaller proportion would enter the eye to exert an effect, though the magnitude of

Fig. 3 Concentration-effect curves for tropicamide-induced mydriasis at 100 min in normal (●) and guanethidine-pretreated (Δ) eye.

the effect produced would be enhanced if these compounds were more potent. Penetration can be quantified very approximately from the tropicamide data if the assumption is made that the concentration of drug in the aqueous humour (volume 0.6 ml) need be no higher than 40 µg/ml to achieve near maximal mydriasis. On this basis, instillation of a 1% solution as a single drop of volume 0.05 ml would provide a total dose of 500 µg, a quantity which is about 20 times greater than that required at most for the pharmacological effect.

Unfortunately drug concentrations in the aqueous humour could not be measured directly, nor can they be deduced from the data obtained, particularly as transcorneal movement of tropicamide is not consistent with simple diffusion (Schoenwald & Smolen, 1971). Preliminary experiments indicate that the log affinity constant (pA_2) of tropicamide for cholinergic receptors of the rabbit isolated iris muscle is 7.04 (Shirley Taylor, personal communication). This finding suggests that the concentrations of the drug applied in the present experiments would be sufficient to produce in the iris acetylcholine dose-ratios of several hundred, were the drug concentrations in the aqueous humour to

Fig. 4 Concentration-effect curves for tropicamide-induced cycloplegia at 100 min in normal (●) and guanethidine-pretreated (Δ) eye.

approach those applied to the eye. In view of this, it seems likely that in the present experiments the equilibrium drug concentrations obtained within the eye were substantially lower than those of the solutions applied.

Within the limits of these considerations, the data illustrate a satisfactory relationship between log concentration of tropicamide and mydriatic and cycloplegic effects produced. Under normal circumstances application of an anticholinergic drug induces mydriasis which is partly sympathetic in origin and the magnitude of the effect produced is presumably modified by progressive removal of sympathetic tone as a reflex response to the light admitted by the dilating pupil. This expectation was fulfilled by the finding that pretreatment with guanethidine reduced the maximum diameter obtainable with cyclopentolate by 0.7 mm and also steepened the tropicamide log concentration-effect curve. Similar considerations would indicate that the slope of the curve would be modified also by progressive increases in parasympathetic tone, likewise reflexly produced. Such a hypothesis could not be examined here.

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