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FACTORS DETERMINING THE POTENCY OF MYDRIATIC DRUGS IN MAN

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1 The mydriasis resulting from topical application of five atropine-like drugs was measured photographically in man. Drug potency was obtained from log dose-response curves.

2 The *in vitro* potency of eight cholinoceptor blocking drugs, including those studied in man, was obtained by measuring their affinity constants for binding to the receptors of an isolated preparation of the rabbit sphincter pupillae. Values agreed closely with those obtained for the muscarinic receptors of guinea-pig ileum.

3 *In vitro* and *in vivo* potency was compared to obtain a quantitative measure of the relative ease with which drugs gain access to the receptors after topical application.

4 The large differences that occur in the intensity and duration of the mydriatic response to atropine-like drugs is primarily the result of differences in their ability to block the receptors. Only with tropicamide does its relatively high accessibility affect its potency in man.

Introduction

There are large differences in the intensity and duration of the pupillary dilatation induced by the clinically used atropine-like mydriatics. Thus atropine and cyclopentolate are used in the therapy of inflammations of the iris because of their powerful and long-lasting effects while weaker drugs such as tropicamide and homatropine are preferred for routine fundal inspection (Mattila, Takki & Indanpaan-Heikkila, 1967; Gambill, Ogle & Kearns, 1967; Smith, 1971). The purpose of this investigation was to determine the factors responsible for these differences in potency.

An important factor is obviously the relative potency of the drugs as antagonists at iris cholinoceptors. This was measured *in vitro* by obtaining their affinity constant (or pA_2) values using an isolated preparation of the rabbit sphincter pupillae and results were compared with values obtained for the muscarinic receptors of guinea-pig ileum.

There are a number of barriers which hinder the access of topically applied drugs to their site of action at the receptors. These include dilution in the tears and aqueous humour and binding to proteins and pigment of the ocular tissues. The most important barrier, however, is poor corneal permeability (Swan & White, 1942). Any difference in the relative potency of mydriatics *in vitro* and *in vivo* is therefore due to differences in their accessibility. A measure of accessibility was made here by comparing *in vitro* with *in vivo* potency obtained from dose response relationships of the drugs in man.

Some of these results were presented to the Physiological Society in 1974 and at the Ninth Pupil Colloquium at Iowa City in 1975.

Methods

In vitro *experiments*

Isolated sphincter preparations were obtained from Chinchilla rabbits (2-4 kg). They were anaesthetized with pentobarbitone sodium and killed by decapitation. The eyes were transferred to a dissection bath where the cornea was pierced within 1 min of death to ensure rapid equilibration with the Krebs-Henseleit solution which was constantly aerated with 95% oxygen/5% carbon dioxide. The cornea was removed and the iris cut circumferentially at a distance of about 2 mm from the pupillary border to obtain a ring of tissue which included the entire sphincter pupillae. The ring was cut across to obtain a strip which was suspended vertically in an organ bath at 37° C. Tissue contractions were measured using an isotonic photoelectric transducer and recorded with a Servoscribe pen recorder. Full scale deflection allowed for maximum contractions and the amplification was approximately x13. A tension of about 100 mg was applied to the tissue via a pulley system in the transducer and friction

was minimized by constant mechanical vibration of the apparatus. As soon as the preparation was set up it always contracted spontaneously and then gradually relaxed over the first hour. No drugs were added until the tissue had relaxed to a steady length.

Pieces of terminal ileum were obtained from albino guinea-pigs which had been stunned and killed by exsanguination. Tissues were suspended in Tyrode solution at 37° C in the same apparatus as that used for the sphincters but with a tension of 1.5 g. A low concentration (2.76×10^{-6} M) of hexamethonium was present.

For both types of tissue carbachol was used as agonist. It was applied every 10 and 2 min for a contact time of 75 and 30 s for the sphincter and ileum respectively. For each concentration of antagonist (B) the log affinity constant (log K) was calculated by the dose ratio method (Schild, 1957) where log K = log (dose ratio − 1) − log B. Only one dose ratio estimation was obtained from each preparation and at least five different concentrations of each drug were tested. Antagonists were allowed to reach equilibrium with the receptors before measurement of dose ratio. The above equation is based on the assumption that the antagonists are competitive. Thus a graph of log (dose ratio-1) against log B yields a slope of unity if this assumption is justified. Such graphs were plotted for all the drugs and the slope of the regression line was calculated by the method of least squares.

In vivo *experiments*

Approval for these experiments was granted by St Thomas's Hospital Ethical Committee. The subject for all the experiments was one healthy hazel-eyed man aged 44 years. Ten drops of each mydriatic solution, made up freshly in 0.15M phosphate buffer pH 6.5, were instilled into the left conjunctival sac at minute intervals. The concentration of this solution was used in the presentation of the results. Before, and at intervals of about 20 min following application, pupil diameters were recorded photographically in duplicate (Sneddon & Turner, 1966) as described by Smith (1971). The subject held a reference cm scale against the upper lip and negatives were projected to a magnification of about x8 for measurement of horizontal pupil diameter. The interclass correlation coefficient between duplicate measurements of pupil diameter was 0.998 showing that the method had a high degree of repeatability. A week was allowed between each drug application to ensure complete recovery. Each drug was tested in about five concentrations.

The peak mydriasis, expressed as a percentage of the maximum attainable (7.75 mm : Smith, 1974) was calculated for each concentration in order to plot log dose response curves. For each drug, the concentration required for 50% mydriasis (EC_{50}) was derived by interpolation from these curves.

Drugs

The following drugs were used: carbachol (CCh, Sigma); (+) and (−)-cyclohexylphenylglycollylethyltriethylammonium iodide ((+) and (−)-CHPGETEA); (±), (+) and (−)-cyclopentolate hydrochloride (Ward Blenkinsop: isomers were prepared by a stereospecific synthetic procedure); hexamethonium iodide (May & Baker); homa-

Table 1 Log K (pA_2) values (± s.e. mean) of cholinoceptor blocking agents

	Rabbit iris sphincter				*Guinea-pig ileum*			
Drug	*log K*	*n*	*Dose ratio**	*Slope†*	*log K*	*n*	*Dose ratio**	*Slope†*
(−)-CHPGETEA	10.16 ± 0.13	5	261	1.34	9.600 ± 0.068**	8	–	–
Atropine	9.00 ± 0.02	10	287	1.03	9.00**	–	–	–
(−)-cyclopentolate	8.61 ± 0.04	5	239	1.08	8.55 ± 0.03	12	897	1.01
(±)-cyclopentolate	8.25 ± 0.04	6	59	0.97	8.00 ± 0.02	13	326	1.02
(+)-CHPGETEA	8.01 ± 0.06	5	83	0.94	7.989 ± 0.035**	7	–	–
Homatropine	7.16 ± 0.03	6	35	1.01	7.39 ± 0.03	10	90	1.04
Tropicamide	7.09 ± 0.02	8	98	1.07	7.14 ± 0.03	10	69	1.09
(+)-cyclopentolate	6.65 ± 0.06	5	17	1.02	6.72 ± 0.02	12	17	0.97

* The value of the highest dose ratio obtained with each drug

† The slope of the plot of log (dose ratio-1) against log B

** Log K values on ileum quoted from Arunlakshana & Schild (1959) for atropine and Barlow *et al.* (1972) for (+)- and (−)-CHPGETEA

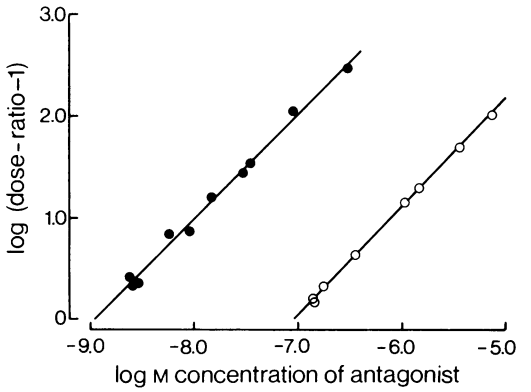

Figure 1 Competitive antagonism of carbachol by atropine (•) and tropicamide (○) on the rabbit isolated sphincter pupillae.

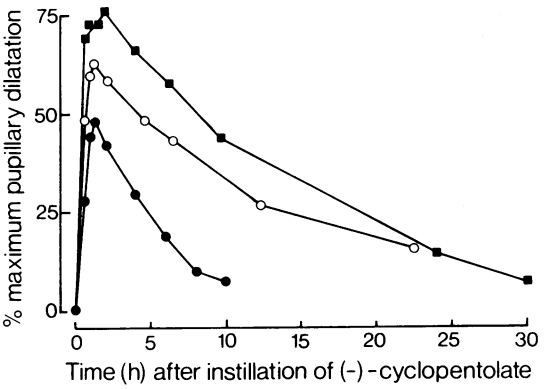

Figure 2 The mydriasis resulting from topical application of (−)-cyclopentolate (• 7.62 x 10^{-5} M; ○ 1.52 x 10^{-4} M; ■ 3.05 x 10^{-4} M), onto the eye of one hazel-eyed subject.

tropine hydrobromide (MacFarlen Smith); tropicamide (Smith & Nephew).

Results

In vitro *experiments*

The concentrations of antagonists tested gave dose ratio values that ranged from one to the ratios shown in Table 1. Estimates of log K were calculated for each dose ratio and the mean values are listed in Table 1 in decreasing order of affinity for the iris receptors. A close correspondence was found between the values of log K for the receptors of the sphincter and ileum (intraclass correlation coefficient was 0.98).

A plot of log (dose ratio-1) against log B was drawn for each antagonist as shown for atropine and tropicamide in Figure 1. The plots were linear with slopes close to unity (Table 1) confirming that these antagonists were acting competitively. The one exception, (−)-CHPGETEA, yielded a plot which was linear but of slope 1.34 (95% confidence limits 1.21-1.47), indicating that log K increased with increasing concentration. This accounts for the large s.e. mean.

In vivo *experiments*

The pupillary dilatation caused by different concentrations of five of the drugs tested *in vitro* was measured following topical application to the human eye. Control experiments established that the drug solvent had no effect on the pupil. Figure 2 shows the results with three concentrations of (−)-cyclopentolate. In each case, the largest diameter reached was used in the construction of the log dose response curves shown in Figure 3. The responses, which mostly lay within the range 15 to 85% of maximum, increased with log dose in an approximately linear fashion. The curve for (+)-cyclopentolate, the weakest drug tested, is rather flattened at high concentrations. This is probably due to the fact that these caused irritation and consequent dilution of drug in the tears produced.

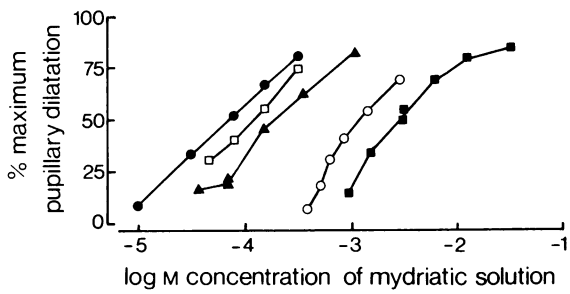

Figure 3 Log dose-response curves for five mydriatic drugs applied to the eye of one hazel-eyed subject. Drugs tested were: (−)-cyclopentolate (•), (±)-cyclopentolate (□), tropicamide (▲), homatropine (○) and (+)-cyclopentolate (■).

Comparison of in vitro *and* in vivo *potencies*

Table 2 shows the drugs that were studied in both situations listed in decreasing order of potency as antagonists at iris cholinoceptors. Mydriatic potency in man, described by the EC_{50} values,

followed the same order except for tropicamide and homatropine. These drugs were approximately equipotent *in vitro* but in man tropicamide was six times more potent than homatropine. The difference is one of accessibility, as shown by the value of the ratios of the *in vitro* and *in vivo* potencies given in Table 2. As tropicamide clearly had the greatest accessibility, that of the other drugs was expressed in relation to that of tropicamide.

Discussion

This investigation has shown that the differential mydriatic potency of the drugs tested here is primarily a function of their ability to block iris cholinoceptors. Only with tropicamide is accessibility an important factor, since its relatively easy access to the receptors makes it more potent *in vivo* than would be predicted from its affinity constant value alone.

The corneal epithelium is the greatest barrier to drug penetration and as it consists of tightly packed layers of cells it is the polar drugs which, in particular, cross the lipid cell membranes with greatest difficulty (Swan & White, 1942; Maurice, 1962). Of all the drugs tested *in vivo* tropicamide is the only one which is mostly nonionized at physiological pH. Thus at pH 7.4 tropicamide is 99% nonionized (pKa = 5.37) while homatropine, for example, is only 0.32% nonionized (pKa = 9.88). It seems likely, therefore, that tropicamide gains access to the receptors more easily than the other drugs because its low degree of ionization leads to a relatively high corneal permeability.

The log K values obtained for the cyclopentolate isomers show that the (−) isomer has an affinity for iris receptors ninety-one times greater than that of the (+) isomer. Therefore the activity of the clinically used drug, the racemate, must be due almost entirely to the (−) isomer. This conclusion is supported by the finding that the affinity of the racemate was approximately half that of the (−) isomer. These differential affinities were reflected in the potency of the drugs *in vivo*.

Since the three forms of cyclopentolate presumably have the same physical properties and hence corneal permeabilities it was surprising to find that their accessibilities were different. There is evidence that some ocular drugs are bound strongly by the pigment of the iris and that this is partly responsible for the relative failure of drugs to act in dark-eyed people (Patil, 1972; Lyons & Krohn, 1973; Patil & Jacobowitz, 1974; Patil, Shimada, Feller & Malspeis, 1974). If cyclopentolate was bound in this way, the bioavailability and hence accessibility of the (−) isomer would be decreased more than that of the (+), not because more of the (−) was being bound (as binding is nonstereoselective, Patil *et al.*, 1974) but because the smaller concentrations of the more potent (−) isomer would be relatively more affected by a saturable binding process. The results of preliminary experiments in which the isomers were tested in light- and dark-eyed people support this hypothesis (Smith, 1975).

These conclusions have been drawn from *in vivo* results with one subject only. This was considered justifiable because the considerable variability in pupillary response to identical drug treatments (Bertler & Smith, 1971) would lead to large errors in EC_{50} estimates obtained from data pooled from a number of subjects.

Barlow, Franks & Pearson (1972) compared affinity constant values for antagonists on the iris and ileum of guinea-pig. As in the present work, they found that the values agreed closely. It can therefore be concluded that the muscarinic receptors of the iris and ileum are of similar structure.

Table 2 A comparison of mydriatic potency *in vitro* and *in vivo*

Drug	*Concentration ($\times 10^{-9}$M) giving a dose ratio of 2 at iris receptors* (a)*	EC_{50} ($\times 10^{-5}$M) in vivo *(b)*	*Accessibility ($\times 10^{-5}$)† (a/b)*	*Relative accessibility (tropicamide = 100)*
(−)-cyclopentolate	2.5	6.8	3.6	8
(±)-cyclopentolate	5.6	11.0	5.1	12
Homatropine	69.2	115.0	6.0	14
Tropicamide	81.3	19.0	42.8	100
(+)-cyclopentolate	224.0	280.0	8.0	19

* This concentration, numerically equal to the reciprocal of K, was taken as an index of *in vitro* potency.
† This ratio of potencies describes the ease with which drugs gain access to the receptors after topical application where the largest values represent the greatest degree of accessibility.

Two of the drugs studies here on the rabbit sphincter, (+)- and (−)-CHPGETEA, were also tested by Barlow *et al.* (1972) on the guinea-pig iris and the log K values obtained for the two iris tissues were identical. The results obtained for (−)-CHPGETEA on the iris were exceptional in that the affinity was so high (ten times greater than atropine) and was significantly different from the ileum value. Further the affinity of the drug was found to increase with increasing concentration and receptor occupancy. If receptors are capable of interacting, an assumption implicit in the cooperative models of drug action (e.g. Changeux, Thiery, Tung & Kittel, 1967), it is possible to speculate on explanations for this observation. It may be that this very potent drug can induce interactions between adjacent receptors of the sphincter such that occupation of one binding site increases the probability of occupation of neighbouring sites. Whatever the mechanism responsible for the increasing affinity, further studies on such potent antagonists would be interesting.

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References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48-58.

BARLOW, R.B., FRANKS, F.M. & PEARSON, J.D.M. (1972). A comparison of the affinities of antagonists for acetylcholine receptors in the ileum, bronchial muscle and iris of the guinea-pig. *Br. J. Pharmac.*, **46**, 300-314.

BERTLER, A. & SMITH, S.E. (1971). Genetic influences in drug responses of the eye and the heart. *Clin. Sci.*, **40**, 403-410.

CHANGEUX, J.-P., THIERY, J., TUNG, Y. & KITTEL, C. (1967). On the co-operativity of biological membranes. *Proc. U.S. Nat. Acad. Sci.*, **57**, 335-341.

GAMBILL, H.D., OGLE, K.N. & KEARNS, T.P. (1967). Mydriatic effect of four drugs determined with the pupillograph. *Arch. Ophthalmol.*, 77, 740-746.

LYONS, J.S. & KROHN, D.L. (1973). Pilocarpine uptake by pigmented uveal tissue. *Am. J. Ophthalmol.*, **75**, 885-888.

MATTILA, M.J., TAKKI, S. & IDANPAAN-HEIKKILA, J.E. (1967). Actions of some autonomic drugs on the human eye. Experiments on medical students. *Ann. Med. exp. Fenn.*, **45**, 412-416.

MAURICE, D.M. (1962). The cornea and sclera. In *The Eye*, ed. Davson, H. pp. 289-368. New York & London: Academic Press.

PATIL, P.N. (1972). Cocaine-binding by the pigmented and nonpigmented iris and its relevance to the mydriatic effect. *Invest. Ophthalmol.*, **11**, 739-746.

PATIL, P.N. & JACOBOWITZ, D. (1974). Unequal accumulation of adrenergic drugs by pigmented and nonpigmented iris. *Am. J. Ophthalmol.*, 78, 470-477.

PATIL, P.N., SHIMADA, K., FELLER, D.R. & MALSPEIS, L. (1974). Accumulation of (−)-[^{14}C]-ephedrine by the pigmented and the nonpigmented iris. *J. Pharmac. exp. Ther.*, **188**, 342-352.

SCHILD, H.O. (1957). Drug antagonism and pA_x, *Pharmac. Rev.*, 9, 242-246.

SMITH, S.A. (1975). *The pharmacology of the cholinoceptors of the rabbit and human iris.* Ph.D. Thesis, University of London.

SMITH, S.E. (1971). Mydriatic drugs for routine fundal inspection. A reappraisal. *Lancet*, ii, 837-839.

SMITH, S.E. (1974). Dose-response relationships in tropicamide-induced mydriasis cycloplegia. *Br. J. clin. Pharmac.*, **1**, 37-40.

SNEDDON, J.M. & TURNER, P. (1966). Adrenergic blockade and the eye signs of thyrotoxicosis. *Lancet*, ii, 525-527.

SWAN, K.C. & WHITE, N.G. (1942). Corneal permeability. I. Factors affecting penetration of drugs into the cornea. *Am. J. Ophthalmol.*, **25**, 1043-1058.

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