FACTORs DETERMINING THE POTENCY OF
CHOLINOMIMETIC MIOTIC DRUGS AND
THEIR EFFECT UPON THE LIGHT REFLEX IN MAN

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1 Television pupillometry was used to measure the effect of six topically applied cholinomimetic
drugs on the resting diameter and light reflex amplitude of the human pupil. Drug potency was
obtained from dose response curves.
2 The tertiary amines arecoline, aceclidine and pilocarpine were considerably more effective miotics
than the choline esters carbachol, methacholine and acetylcholine.
3 All the drugs which caused miosis also reduced light reflex amplitude proportionally.
4 The in vitro potency of these drugs was also measured on preparations of rabbit iris sphincter
and guinea pig ileum.
5 Dose response relationships for pilocarpine in man and in vitro showed evidence of partial agonist
activity on the rabbit iris only.
6 A comparison of the in vivo and in vitro results showed that three factors influenced the potency of
topically applied miotics: accessibility to the iris; sensitivity to cholinesterase; and cholinoceptor
agonist potency.

Introduction

Cholinomimetic drugs are used widely in ophthalmology for the treatment of glaucoma and in
diagnostic tests for drug supersensitivity resulting from parasympathetic denervation of the pupil
(Thompson, 1975). There have been few detailed studies on the ocular pharmacology of these drugs,
most work being confined to single concentrations of the more commonly used ones (Lowenstein &
Loewenfeld, 1953; Ogle, Whisnant & Hazeldrig, 1966). The present work involved dose response studies on
six cholinooceptor agonists in man and on preparations of rabbit iris and guinea pig ileum in vitro. Potency
values obtained in vivo and in vitro were compared to obtain a measure of the accessibility of the drugs to
the iris following topical application to the cornea.

Drug effects on both the resting pupillary diameter and on the size of the reflex constriction to a light
stimulus were recorded by means of television pupillometry. It is known that the light reflex is reduced by
mydriatic drugs (Gambill, Ogle & Kearns, 1967) and by the miotic pilocarpine, an effect that has been
attributed to its partial agonist activity (Newsome & Loewenfeld, 1974). This study examined whether full
agonists also inhibited the light reflex. Some of these results were presented in 1977 to the British
Pharmacological Society (Smith & Smith, 1977) and to the Tenth Pupil Colloquium in New York.

Methods

In vivo experiments

Approval for these experiments was granted by St.
Thomas’s Hospital Ethical Committee. The subject of
all the tests was a healthy hazel-eyed female aged 25
years. Ten drops of each drug solution, made up
freshly in 0.15 M phosphate buffer pH 6.5, were instilled into the right conjunctival sac at 1 min
intervals. The concentration of this solution was used
in the presentation of the results. The interval between
experiments was at least 1 week and each active drug
was tested in four or five concentrations.

Pupillary measurements were made with a
Whittaker Corporation Series 1800 binocular infra-
red television pupillometer (Lowenstein & Loewenfeld,
1958) before and at intervals of 5–10 min following
drug application. Measurements were performed in
darkness but the subject was not dark-adapted. Trains
of six Maxwellian light stimuli of 0.5 s duration,
foocussed in the plane of the pupil, were directed at
the untreated eye and were far enough apart (10 s) to
allow complete recovery between each reflex pupillary
constriction. The intensity of the stimuli gave
responses of about 60% of the maximum for this
subject. Responses of both pupils were recorded, the
second to sixth responses inclusive being averaged to yield the amplitude of the reflex. The average resting diameter was derived from the foot of each reflex.

Peak miosis, expressed as the difference in diameter between the treated and untreated eyes, at each concentration was used to construct log dose response curves. For each drug, the EC$_{50}$ (where 50% miosis was 3.5 mm in this subject) was derived by the method of least squares from the straight-line portion of these curves. The mean molar potency ratios relative to carbachol (EC$_{50}$ carbachol/EC$_{50}$ test drug) were calculated.

Drug effects on the light reflex were expressed as the left-right difference in reflex amplitude as a percentage of that of the untreated left eye (since the direct and consensual reflex responses are of equal size).

**In vitro experiments**

Isolated preparations of sphincter pupillae from New Zealand White rabbits and terminal ileum from albino guinea pigs were prepared as previously described (Smith, 1976). Each drug was tested in a range of concentrations on both tissues and log dose-response curves constructed. Mean molar potency ratios of at least five estimates (carbachol = 1) were obtained using a standard four-point assay technique.

**Drugs**

The following drugs were used: aceclidine (3-acetoxy-quinuclidine) hydrochloride (Chibret); acetylcholine chloride (ACh, Lematte & Boinot); arecoline hydrobromide (Sigma); carbachol (CCh, Sigma); eserine sulphate (T & H Smith); methacholine chloride (MeCh, Sigma); pilocarpine nitrate (Smith & Nephew).

The partition coefficients (three estimates) of some of these drugs between toluene and 0.15 M phosphate buffer pH 6.5 were measured following equilibration with shaking for 15 min at 20°C. The aqueous phase was retrieved by centrifugation and drug concentration in buffer before and after partition was measured from appropriate dilutions by four-point assay on isolated guinea pig ileum.

**Results**

Of the six cholinomimetics tested, only the three tertiary amines, arecoline, aceclidine and pilocarpine, produced full miosis in man (Figure 1). The choline esters had little activity: CCh produced some miosis but ACh and MeCh were ineffective at tolerable concentrations. The curve for CCh was flatter than those obtained with the three more potent drugs, probably because the high concentrations needed were irritant and so caused dilution of the drug in the tears produced. Potency ratios for the tertiary amines relative to carbachol (Table 1) were therefore slightly higher than would have been obtained with exact parallelism.

Arecoline was found to have a faster time course than the other drugs, reaching half-maximal miosis in 25 min compared with 1 h taken by the other miotics.

The drugs which caused miosis also reduced the size of the light reflex proportionally (Figure 2), the relationship being independent of the particular miotic used.

The response of the rabbit iris in vitro to these cholinomimetic drugs is illustrated in Figure 3. These curves are drawn from single typical experiments but at least five experiments were performed for determination of potency ratios (Table 1). Four of the six drugs tested gave full, parallel dose response curves.
Figure 3  Log dose-response curves for contraction of the isolated rabbit iris sphincter with ACh (▲), pilocarpine (▼), aceclidine (△), arecoline (▲), MeCh (□) and CCh (■). ACh (●) and MeCh (○) were also tested in the presence of eserine (6.2 x 10^-6 M).

but that for pilocarpine was typical of a partial agonist with a maximum contraction of only 20% that obtained with the other drugs. The curve for ACh was flatter than those for the other full agonists but in the presence of eserine, at a concentration that completely inhibits iris cholinesterase (Smith, 1975), it was parallel to the others. The accompanying shift to the left indicated that a considerable potentiation had occurred (ratio of EC50 values = 1370 ± 775). Similarly, the potency of MeCh was increased by 22 ± 5-fold with eserine. All the drugs, including pilocarpine, gave full parallel dose response curves when tested on guinea pig ileum.

Table 1 shows the mean molar potency ratios in vitro and in vivo. The order of potency of the three full miotics in vivo corresponded with that found in vitro. Potency ratios for arecoline and aceclidine agreed well between iris and ileum but the ratios for the cholinesterase-sensitive esters, MeCh and ACh, were far lower on rabbit iris. No ratio was obtained for pilocarpine on rabbit iris because of its partial agonism on this tissue.

Values for the relative accessibility of these drugs to the iris receptors following their topical application in man are shown in Table 2, together with the pK_a values, percentage of drug in the non-ionized form and

Table 1  Mean ± s.e. mean molar potency ratios (CCh = 1) for cholinomimetic drugs

<table>
<thead>
<tr>
<th></th>
<th>In vivo miosis</th>
<th>In vitro rabbit iris sphincter</th>
<th>In vitro guinea pig ileum</th>
</tr>
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<tbody>
<tr>
<td>Arecoline</td>
<td>350</td>
<td>0.29 ± 0.02</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>Aceclidine</td>
<td>87</td>
<td>0.14 ± 0.006</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>61</td>
<td>—</td>
<td>0.09 ± 0.006</td>
</tr>
<tr>
<td>CCh</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MeCh</td>
<td>0</td>
<td>0.23 ± 0.04</td>
<td>1.40 ± 0.08</td>
</tr>
<tr>
<td>ACh</td>
<td>0</td>
<td>0.014 ± 0.005</td>
<td>1.49 ± 0.13</td>
</tr>
</tbody>
</table>

Table 2  Relative accessibility, pK_a*, % non-ionization and toluene: buffer partition coefficient of miotic drugs

<table>
<thead>
<tr>
<th>Relative accessibility (carbachol = 1)*</th>
<th>pK_a</th>
<th>% non-ionized at pH 6.5</th>
<th>Toluene: buffer partition coefficient at pH 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arecoline</td>
<td>720</td>
<td>7.6</td>
<td>7</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>670</td>
<td>7.1</td>
<td>21</td>
</tr>
<tr>
<td>Aceclidine</td>
<td>460</td>
<td>4.9</td>
<td>98</td>
</tr>
<tr>
<td>CCh</td>
<td>1</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

* This ratio of in vivo/in vitro (ileum) potency ratios describes the ease with which drugs gain access to the iris receptors with the largest values representing the greatest degree of accessibility.
the lipid solubility, represented by the partition coefficient, at the pH of the buffer in which the drug was applied.

Discussion

The present investigation has shown that the three tertiary amines studied were considerably more effective miotics than the choline esters. Arecoline, the most potent drug tested, was active at a concentration as low as 0.0025%. The high potency and fast time course of this lipid soluble drug would make arecoline particularly well suited for diagnostic drug tests. Although once used in ophthalmology, its popularity declined since its transient action made it unsuitable therapeutically. Aceclidine, a synthetic substance used in some European countries for glaucoma therapy, was found to be of similar potency to the frequently used pilocarpine, a finding which corresponds to their equiactivity as ocular hypotensive agents (Romano, 1970).

Due to the difficulty of obtaining subjects willing to participate in such a prolonged study (1 year), dose response curves were obtained from only one subject. The potency ratios in man must therefore be regarded as estimates and not absolute values.

The results have indicated three factors to be important determinants of miotic potency in man. The first is that of accessibility to the iris following topical application. The choline esters, which were active in vitro but virtually ineffective in vivo, evidently had more difficulty crossing the cornea than did the tertiary amines. This may not seem surprising since the choline esters, in contrast to the tertiary amines, are fully ionized. However, the results in Table 2 showed that there was no simple relationship between accessibility and the degree of ionization or the lipid solubility. The three tertiary amines had similar accessibility values despite a 14-fold difference in ionization and a 50-fold difference in solubility. Although corneal lipid solubility is not necessarily reflected accurately by partition coefficient values, the work of Seeman (1972) suggests that they can at least predict the rank order of biological lipid solubilities.

The second factor of importance is that of sensitivity to cholinesterase. Of the three choline esters, only CCh, which is resistant to the enzyme, had any action in man. The influence of cholinesterase in the iris is obviously very marked since ACh and MeCh were greatly potentiated by eserine in vitro.

Not surprisingly, the third factor contributing to miotic potency was their relative activity as cholinoreceptor agonists. Similarly, the mydriatic potency of drugs with comparable accessibility values could be predicted from their activity as cholinoreceptor antagonists (Smith, 1976).

Pilocarpine, a cholinoreceptor agonist known to be of low efficacy (Furchgott & Bursztyn, 1967), was obviously a partial agonist on the isolated rabbit iris despite the fact that the shape of its dose response curve on the human iris (in vivo) and on guinea pig ileum was apparently no different from that of the other active drugs. Similarly, van Rossum (1960) has demonstrated that pilocarpine acts as a pure agonist, partial agonist, or antagonist when tested on different tissues. In terms of drug receptor theory, the difference between tissues in their response to pilocarpine must represent variations in efficacy and/or spare receptor reserve.

The amplitude of the light reflex was reduced by all the drugs studied, the effect being entirely dependent on the amount of pupillary constriction. To date, among miotics only pilocarpine has been reported to reduce reflex size in this way (Morgan, Hollenhorst & Ogle, 1968; Newsome & Loewenfeld, 1974). Newsome & Loewenfeld suggested that the effect might be related to the partial agonist activity of pilocarpine, an explanation which now seems unlikely since the full agonists tested here also reduced reflex size. An alternative explanation becomes apparent from a consideration of the relationship between drug effect and concentration. Drug effect (decrease in pupillary diameter) increases hyperbolically, not linearly, with increasing agonist concentration (of ACh released during a reflex response or of miotic instilled). Thus although the same amount of ACh may be released during a reflex before or during miotic action, in the latter case the ACh is acting further up the hyperbolic dose response curve, and thus the amount of reflex constriction is necessarily reduced. It thus appears that cholinoreceptor agonists and antagonists, which constrict and dilate the pupil respectively, each cause a reduction in the size of the light reflex.

This work was supported by the Medical Research Council, the Prevention of Blindness Research Fund and the Royal National Institute for the Blind. Aceclidine was kindly supplied by Chibret Laboratories.
References


(Received August 16, 1977)