
A rapid and precise method for evaluating the miotic activity of cholinergic drugs has been developed based on Long's method for measuring the rate of mydriasis. The rate of reversal of mydriasis developed previously in the intact mouse eye by a mild mydriatic (phencyclidine) is used to evaluate the miotic activity. The method provides a useful tool for measuring and comparing the miotic activity of acetylcholine agonists and cholinesterase inhibitors.

Measuring the change in the diameter of the pupil in response to certain autonomic drugs is a well-known method whereby several parameters of the drug effect can be evaluated, namely, its onset period, rate of action, magnitude, and duration.

In order to measure accurately a miotic response, however, one has to eliminate the disturbing light reflex. A simple way involves only the use of a transparent ruler in a dimly lighted room, but this method is not ideal, mainly because it is difficult to measure accurately in the dim light needed to eliminate most of the light reflex.

Several types of pupillometers were developed during the last three decades, but none were able to provide highly accurate measurements totally without light reflex, save for the complex infra-red electronic pupillograph constructed by Loewenstein and co-workers.

In the present communication, we present a simple, accurate method for measuring miotic and mydriatic pupillary responses in the mouse eye which completely eliminates the light reflex. The method is based on that previously described by Armaly and Long.

Materials and methods. Phencyclidine-HCl [1(1-phenylcyclohexyl)piperidine] was prepared according to Kalir and co-workers. Atropine (sulphate, hydrate) and eserine (physostigmine) in the salicylate form were obtained from Sigma Chemical Company. Mydriaticum (tropic amide, N-ethyl-N-pyridyl-4-methylpropamide), cyclopentolate [2-dimethylnaenoxyethyl-N-(1-hydroxycyclopentyl)-N-phenyl-acetate] and phospholine iodide (euchothiolate, S-ester of (2-mercaptopethyl)-triamethyliummonium iodide with 0,0-diethylphosphorothionate) were ordinary ophthalmic solutions. Arecoline-Br and oxotremorine were obtained from Altrich and DFP (disopropylphosphorofluoridate) from Fluka; DMAEA (2-acetoxy-1-dimethylaminoethanol) and acetylcholine were synthesized by acylation of the appropriate amino alcohol with acetic anhydride in pyridine.

A mouse is placed under a binocular microscope (Nikon, ×40). The eye of the mouse is sharply focused and the length of the horizontal diameter of the whole eye and the pupil are accurately read by one observer, using a scale located between the ocular (×20) and the objective (×2). The scale contains 180 divisions per centimeter. The light source was kept at a fixed distance of 10 cm. from the eye of the mouse. The illumination source (6 volts, 5 amperes) was obtained from Eliza (Tokyo). Illumination was kept constant by the use of a transformer (Eliza, Tokyo). A drop (ca. 50 μl) of 10-4 M phencyclidine in 0.1 M phosphate buffer, pH 7.8, is then placed carefully on the eye of the animal without touching the cornea and wiped out gently 20 seconds later. After an interval of more than three minutes, the diameter of the pupil is again measured under the microscope. The 50 μl drop covers the surface of the eye, and its excess was removed quantitatively at the appropriate intervals simply by absorption with a piece of cotton without touching the cornea. This procedure which did not influence the diameter of the pupil was preferred over washing with, e.g., saline, since the latter can cause a damage to the corneal epithelium.

Those animals having a pre-experimental pupil diameter more than 25 per cent of the whole eye diameter or mice which did not develop a mydriasis of at least 80 per cent of the whole eye, are rejected. These criteria eliminate about 20 per cent of the tested animals. The population of rejected animals was not influenced by age or sex.

A drop of the miotic agent is then applied to the eye and the rate of the reversal of the mydriasis is measured every 30 seconds.

To study an effect of a mydriatic drug after the initial measurements of the diameters of the whole eye and the pupil, a drop of the drug is applied to the eye and the development of mydriasis is followed continuously, according to Armaly and Long. The interval between readings depends on the rate of onset and the duration of the effect. Pupillary change is expressed as percentage of the pupil diameter of the whole eye. Values presented throughout this report are
the mean of at least six experiments, carried out by one observer, on six different animals.

Reproduction of results was checked by taking six successive pupillary diameter readings of the resting pupil under the conditions of strong and constant illumination described above, and six successive different readings of the diameter of a maximum mydriatic pupil. Measurements were carried out at 15-second intervals by one observer. Diameter of the resting pupil expressed as percentage of the pupil diameter of the whole eye was found to be 20 ± 2 per cent scale units (S.E.M.). Diameter of the pupil at the maximum obtainable mydriasis expressed as percentage of the pupil diameter of the whole eye was found to be 90 ± 2 per cent scale units (S.E.M.). It should be noted that similar results were obtained when several observers participate in the measurements.

Results and discussion. The method of comparing the miotic activity of drugs is based on competition between the miotic drugs tested and the mild mydriatic effect of phencyclidine in the cholinergic system of the sphincter of the intact unine eye. The rate of reversal of the previously developed mydriasis is measured under standard defined conditions.

The optimal mydriatic agent should induce a

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Fig. 1. Onset (a) and duration (b) of mydriatic activity of clinically used mydriatic drugs: Δ—Δ atropine sulphate (10⁻¹ M), ○—○ mydriaticum (5 x 10⁻⁴ M), •—• cyclopentolate (3 x 10⁻³ M), and □—□ phencyclidine (10⁻¹ M). Each point represents the mean results of experiments on six animals. Horizontal bars denote standard error of the mean. 1. Time of drug application.
Fig. 2. Rate of reversal of previously developed mydriasis by $10^{-2}$ M arecoline. One drop of the drug placed on the mouse eye: full mydriasis had been developed previously by cyclopentolate ($3 \times 10^{-4}$ M), mydriaticum ($6 \times 10^{-4}$ M), or phencyclidine ($10^{-3}$ M). Each point represents the mean results of experiments on six animals. Horizontal bars denote standard error of the mean.

full and rapid mydriasis while having a sufficiently low affinity to the cholinergic receptor to permit efficient competition by the miotic drugs. In the search for optimal performance, the mydriatic activities of atropine, cyclopentolate, and mydriaticum at the minimal concentrations needed to induce full mydriasis in the mouse eye were compared to that of phencyclidine.

A measure of the relative affinity of the drugs to the sphincter cholinergic receptors may be obtained by determining both the minimal concentrations at which maximal mydriasis may be induced and the duration of mydriatic activity. As shown in Fig. 1, phencyclidine activity had the shortest duration (the plateau, 15 minutes), while atropine activity persisted for more than two hours. The time of onset of complete mydriasis for mydriaticum and cyclopentolate is similar to that for phencyclidine, around 3.4 minutes—while the time of onset of atropine activity is about 15 minutes. The relative anticholinergic potencies of the drugs may also be compared by competing them with a cholinergic drug on the same receptor. One drop of $10^{-2}$ M arecoline (in 0.1 M phosphate buffer, pH = 7.4) was applied locally to the same eye in which mydriasis had been induced previously by one of the mydriatic drugs. The rate of the reversal of the mydriasis caused by the miotic agent was measured using the technique described above (Fig. 2). Atropine was omitted in this experiment due to its slow action, the long duration of its effect, and its high affinity to the receptor—evidenced by the low concentration needed to induce full mydriasis.

The mydriasis developed by phencyclidine (Fig. 2) was antagonized by arecoline faster than that developed by either of the other two anticholinergic drugs.

From the data presented in Figs. 1 and 2, it seems that among the four anticholinergic drugs tested, phencyclidine has the lowest affinity to the cholinergic receptors in the sphincter muscle; both a relatively high concentration is needed to develop full mydriasis ($3 \times 10^{-3}$ M), and its mydriatic activity is of short duration (15 minutes). Indeed, the affinity constant of phencyclidine to a peripheral muscarinic receptor was found to be lower by three orders of magnitude than that of atropine.16

The sensitivity of the mouse eye to phencyclidine is not influenced by age (among the age group of 3 to 6 months) or sex of the animals (see also methods).

The effect of the contact time (see methods) of phencyclidine solution with the mouse eye on the time of onset of mydriasis was measured (Figs. 3 and 4). The rate of the developing mydriasis after contact times of five and twenty seconds is shown in Fig. 3 and data are presented in Fig. 4 for the five different contact times measured. Increasing the contact time from 5 to 40 seconds shortened the time needed for the
Fig. 3. Effect of contact time of phencyclidine (HCl) solution (10^{-2} M in 0.1 M phosphate buffer, pH = 7.4) on the rate of the developing mydriasis. See text for experimental details. •—• 5 seconds, O—O 20 seconds. Each point represents the mean results of experiments on six animals. Horizontal bars denote standard error of the mean.

Fig. 4. Effect of increasing the contact time of phencyclidine (HCl) solution with the mouse eye on the time needed for development of full mydriasis (T_{max}). Each point represents the mean results of experiments on six animals. Vertical bars denote standard error of the mean.

development of full mydriasis from five to two minutes, the minimal onset time obtainable (Fig. 4). A 20-second contact time was chosen as optimal for all further experiments.

On the basis of these experiments, the following standard conditions were chosen for developing mydriasis in order to evaluate the miotic activity of cholinergic drugs: (1) the duration of a single test is 10 minutes, which is the duration of full mydriasis induced by 10^{-2} M phencyclidine in 0.1 M phosphate buffer, pH 7.8; and (2) the contact time of the phencyclidine solution with the mouse eye is 20 seconds.

The relative miotic potencies of some well-known cholinergic drugs as determined by this method are summarized in Table I. Three minutes after phencyclidine treatment, one drop of the drug to be tested, in buffered solution, was applied to the treated eye and the pupil diameter was measured. Comparative values of miotic activity are obtained by determining the time needed for reversal of an arbitrarily selected percentage of the previously developed mydriasis, e.g., 50 per cent (T_{50}) (Table I).

There are several biological parameters which might influence the overall biological response of
Tel-Aviv University, Tel-Hashomer, Israel. Sub-

Drugs.

mitted for publication Sept. 26, 1974. Reprint

Key words: miotic activity, mouse eye, cholinergic

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Key words: miotic activity, mouse eye, cholinergic

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Intact omentum for ocular vascularization.

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AND PETER V. PALENA.

Twenty dogs had their intact omentum exten-
sively lengthened by a series of surgical maneu-
vers. Transverse incisions were then made along
the chest, shoulder, neck, and scalp which were
undermined and connected to form a subcutaneous
channel through which the omentum was brought
up to the orbit. The lateral rectus muscle of the
eye was divided and a septal flap developed along
the lateral superior region of the eye which ex-
posed the choroid upon which the omentum was
secured. Subsequent studies demonstrated vascular
connections between intraocular vessels and those
of the omentum. Proof of the existence of these
vascular connections was based upon fluorescent
funduscopic, gross, and histologic evidence.

The intact omentum has been used surgically
for a variety of clinical problems. It has been
recently shown in our laboratory that the intact
omentum can be successfully transposed to the
brain and spinal cord of the dog. The purpose

Table I. Reversal of activity by cholinergic drugs
acting on previously developed mydriasis in the
intact mouse eye

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration* (M)</th>
<th>%50% Reversal time (sec) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Acetylcholine-lik:</td>
<td></td>
<td></td>
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<tr>
<td>Arecoline</td>
<td>10^-2</td>
<td>53 ± 1.6</td>
</tr>
<tr>
<td>Oxotremorine</td>
<td>10^-2</td>
<td>203 ± 5.4</td>
</tr>
<tr>
<td>Aceticholine</td>
<td>10^-2</td>
<td>354 ± 14.3</td>
</tr>
<tr>
<td>DMAEAI</td>
<td>2 × 10^-2</td>
<td>397 ± 7.5</td>
</tr>
<tr>
<td>II. ChE inhibitors:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eserine (salicylate)</td>
<td>10^-2</td>
<td>94 ± 4.6</td>
</tr>
<tr>
<td>Physostigmine (iodide)</td>
<td>1.6 × 10^-3</td>
<td>125 ± 17.3</td>
</tr>
<tr>
<td>DFP§</td>
<td>10^-3</td>
<td>303 ± 7.6</td>
</tr>
</tbody>
</table>

One drop of the mydriatic drug was applied locally to the intact mouse eye which was kept under strong and constant illumination. The pupil diameter was measured by a binocular microscope (x40) every 30 seconds.

*a In 0.1 M phosphate buffer, pH = 7.0.

Time needed for reversal of the usually 90 per cent developed mydriasis (from 20 per cent) to (1 - r/2)/20 = 55 per cent mydriasis.

§ Dimethylaminoethylacetate, the tertiary analog of acetylcholine.

Concentration" (M)

The skillful technical assistance of Miss R. Calron is gratefully acknowledged.

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Key words: miotic activity, mouse eye, cholinergic drugs.

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