Hydrogel polymer lenses may have overloaded a carrier mechanism. When overloaded this way, the cornea appears to be able to take up pilocarpine but incapable of transporting it quantitatively into the aqueous. Unless transported further without delay, retained corneal pilocarpine does not appear in the aqueous analog as the intact molecule, whatever its eventual fate. In contrast, depending on its structure and elution resistance, the hydrogel polymer lens in the presence of tear flow appears to expose the cornea to a capacity pilocarpine load which can be handled by the proposed carrier mechanism without a "reservoir" effect.


Key words: pilocarpine transport, isolated cornea, hydrogel polymer, UV spectroscopy, corneal transport, tear elution.

References


Differential effects of inhibitors of monoamine oxidase Types A and B on the adrenergic system of the rabbit iris. E. A. Zeller, Paul A. Kneipper, and David Shoch.

Topical instillation of inhibitors of monoamine oxidase Types A or B into the rabbit eye, followed by the administration of a releaser of monoamines (Ro 4-1284), induces strong and sustained dilation of the iris. The mydriatic pattern differs markedly with A- and B-inhibitors and with the duration of the inhibitor treatment. The Type B inhibitor, deprenyl, presents the degradation of exogenous 2-phenylethylamine and possibly of the endogenous amine X capable of releasing monoamines from a compartment less accessible to Ro 4-1284.

In a series of classical studies the participation of the α-adrenergic system in the stimulation of the dilator iridis has been clearly demonstrated. Our knowledge of the function of the various components of this system, however, is still quite limited. For one, there are differences of opinion as to whether monoamine oxidase (MAO, E.C. 1.4.3.4.) is a mere scavenger of straying monoamines or whether it is an essential part of the apparatus establishing and maintaining optimal distribution and concentration of monoamines. We report here some studies designed to find out whether MAO exerts a controlling influence in iris movements. We started with the following observation: the instillation of the inhibitor pergolide in the rabbit eye markedly increased the level of monoamines in the iris and decreased the formation rate of the aqueous humor, but it did not affect the pupillary diameter. 1 2 3: The monoamines pentup by MAO blockade, however, can be made functionally visible by their hydric action, when the monoamine releaser Ro 4-1284, a benzoxazinoline derivative, is instilled. Thus, it became possible to investigate the efficiency of MAO inhibitors in the intact eye. It has been recently shown that two types of MAO's exist, Types A and B, which apparently play different metabolic roles in amine metabolism. In vivo, Type A preferentially attacks the neurotransmitters norepinephrine (NE) and serotonin, while phenylethylamine appears to be one of the physiologic substrates of Type B. 4 5 Several classical MAO inhibitors show preference for...
Fig. 1. Effect of 0.004 M clorgyline (A), deprenyl (B), Lilly 51641 (A, B), pargyline (A>B), and 0.001 M Su 11,739 (A, B) on pupillary diameter after two and 72 hours of treatment prior to the administration of Ro 4-1284.

Animals and methodology. Five to six New Zealand albino rabbits weighing 4 to 6 kilograms were used in each experiment and received all drugs by topical conjunctival instillation. We restrained the animals by wrapping them in cotton cloths. Pupil measurements to the nearest half millimeter were obtained with calipers in standard room illumination of 15 foot-candles. We present the results here as millimeters of difference (Δ) between iris diameters of the treated and untreated eyes. For testing the direct inhibitor action, we gave one drop of a MAO inhibitor at 10-minute intervals for three doses and then recorded measurements. For the evaluation of indirect action, we followed the same procedure and released the monoamines with Ro 4-1284 two hours after the administration of the last inhibitor drops. In long-term experiments, a total of nine drops of the MAO inhibitors were spread over three days prior to the testing period and two hours after the last application of the inhibitor, the monoamines were released with Ro 4-1284. This compound was given in 10-minute intervals for three doses. To block α-adrenergic receptors, we instilled a one percent solution of the thymoxamine 15 minutes for two doses prior to the amine release by Ro 4-1284. All drugs were dissolved in 0.067 M phosphate buffer, pH 7.2. For the determination of the rabbit MAO activities, we prepared homogenates from the combined iris and ciliary body (which, when prepared from the rabbit eye, are difficult to separate). This “iris” homogenate was incubated in phosphate buffer at pH 7.2 to 7.8 for 30 to 60 minutes. To follow the degradation of 10⁻¹⁰ M 5-methoxytryptamine (5-MTA) in the presence of 10⁻¹⁰ M semicarbazide, the increase of optical density at 240 nm, was taken from the sequence of recorded absorption spectra. The other main substrate, 10⁻⁶ M α-aminophenylethlamine (α-APE), by the action of Type B MAO, is converted into indole which is assessed colorimetrically at 625 nm.²

On the occurrence of types A and B in the rabbit iris. MAO Type B displays a very high specificity for α-APE. In fact, we have not been able to observe any clear-cut degradation of this amine by MAO preparations of high A activity. This amine was oxidized by iris homogenates at a rate of 3.9 amoles per hour per gram of fresh tissue, as compared with 52 and 0.4 amoles for rabbit liver and basal ganglia, respectively. When deprenyl which has a higher affinity for the B-type than any other inhibitor, was instilled in vivo into the conjunctival sac, at 0.004 M solution, no degradation of α-APE could be observed. However, homogenates obtained from equally treated eyes still were capable of oxidizing 5-MTA,
Fig. 2. Effect of combined action of 0.004 M deprenyl (B) and Lilly 51641 (A, B) for 72 hours of treatment prior to the instillation of Ro 4-1284. The observations are given in terms of Δ mm. ± S.E. Dark bars: deprenyl, crosshatched bars: Lilly 51641, dotted bars: both inhibitors.

at 0.02 amoles per hour per gram, a substrate with a high preference for the A-type. This activity disappeared after in vivo instillation of clorgyline, the most typical Type A inhibitor. Thus, both types of MAO appear to be present in the iris.

Direct mydriatic responses to the amine releaser Ro 4-1284 and to various MAO inhibitors. The topical instillation of 0.01 M of the benzquinazoline produced a small (Δ = 0.4 mm.) and statistically insignificant mydriasis. All the MAO inhibitors, used in these experiments at 0.004 M concentrations (0.001 M Su 11,739) did not produce any pupillary changes when applied alone.

Release of monoamines after MAO inhibition. When we instilled MAO inhibitors for two hours prior to the administration of the releaser, a considerable protection of the releasable monoamines could be expected. This was indeed observed (Fig. 1). The inhibitor treatment of the iris led to a monophasic mydriasis with the largest opening of the pupil occurring between 20 and 30 minutes. The range of pupillary reactions varied markedly after the instillation of clorgyline (A) and of deprenyl (B), while the inhibitors affecting both MAO types occupy an area between the two other agents. After 72 hours of inhibitor action the mydriasis was either increased with Su 11,739 (A, B) and deprenyl (B), or decreased with Lilly 51641 (A, B), or remained approximately the same with clorgyline (A). In addition, the response pattern to the inhibitors affecting both types of MAO changed drastically. A second reaction phase followed the first one, reaching the highest reading after two hours.

Upon the administration of the α-blocker thymoxamine, the mydriasis seen after 72 hours of clorgyline pretreatment was abolished. This observation fits into the general concept that the Type A of MAO is capable of inactivating norepinephrine and that clorgyline preferentially inactivates this MAO type.

To get some more information regarding the differences in mydriatic patterns described above, we combined the administration of deprenyl (B) with that of other inhibitors, e.g., Lilly 51641 (A, B) (Fig. 2). The resulting pupillary movement caused by the drug combination is much stronger than the sum of the individual reactions. The outcome of this and other combination experiments indicated that the blocking of the
studied the pupillary influence of a number of a preferential substrate of MAO Type B, goes of norepinephrine and that the level of another amine (compound X) which, if it exists, must lie hardly be clue to the protection and accumulation of norepinephrine.

In looking for this hypothetical X, we have studied the pupillary influence of a number of amines, including 2-phenylethylamine. This monoamine is known to be an efficient releaser of monoamines. Its topical administration does not induce mydriasis. When the eye, however, is pretreated with a MAO inhibitor, the degree of 2-phenylethylamine-induced mydriasis is determined by the type of inhibitor (Table 1). This observation again demonstrated the enormous efficiency of MAO located in the iris. It also made it feasible that the type of inhibitor used, A or B, can be recognized by simple pupillary measurements. Furthermore, we measured the level of 2-phenylethylamine in rabbit iris by gas chromatography and found an unusually high level of this amine, ranging from 5 to 10 µg per gram of tissue. (Mosnaim, A. D., Huprikar, S. V., Borison, R. L., et al.: unpublished data.) With the same method, the level of this amine was determined to be 0.7 to 1.3 µg per gram. These observations strengthen our hypothesis that 2-phenylethylamine plays some role in the physiology of the rabbit iris.

From the experiments reported here we can deduce that MAO activity of the iris can influence the pupillary response to perturbations of the metabolism of endogenous and exogenous monoamines. The results favor the concept of MAO as being an integral part of the iris adrenergic system. They also suggest that two compartments containing α-adrenergic agents exist. One compartment releases its monoamines in the first reaction phase upon instillation of Ro 4-1284, while the second compartment is more resistant to this release. It is forced, however, to release its content in a second phase when substance X, supposedly a physiologic substrate of MAO Type B, is accumulating. Thus, a sort of cooperation between the two MAO types begins to emerge. It would be a novel form of regulation of a sustained mydriasis which is not due to a primary, but to a secondary release of norepinephrine.

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Key words: adrenergic system of the iris, clorgyline, deprenyl, monoamine oxidase inhibitors, monoamine releaser Ro 4-1284, monoamine oxidase types A and B, pargyline, 2-phenylethylamine.

REFERENCES


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### Table 1. Effect of 0.01 M 2-phenylethylamine on the pupillary diameter in controls and in the eyes treated for 72 hours with various inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Peak (min.)</th>
<th>Duration (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.8</td>
<td>30</td>
</tr>
<tr>
<td>Clorgyline (A)</td>
<td>4 mM</td>
<td>4.3</td>
</tr>
<tr>
<td>Sm 11,739 (A + B)</td>
<td>1 mM</td>
<td>4.4</td>
</tr>
<tr>
<td>Deprenyl (B)</td>
<td>4 mM</td>
<td>4.3</td>
</tr>
</tbody>
</table>

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-methylpropionamide), cyclopentolate [2-dimethylaminoethyl-α-(1-hydroxycyclopentyl)-α-phenyl-acetate] and phospholine iodide (echotiolate, S-ester of (2-mercaptoethyl)-trimethylammonium iodide with 0,0-diethylphosphorothioate) were ordinary ophthalmic solutions. Arecoline-HBr and oxotremorine were obtained from Altrich and DFZ (disisopropylphosphorofluoridate) from Fluka; DMAEA (2-acetoxy-1-dimethylaminoethanol) and aceclidine 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 lengths of the horizontal diameter of the whole eye and the pupil are accurately read by one observer, using a scale located between the ocular (+30) and the objective (×2). The scale contains 100 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 10⁻² 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...