Enzymology of the refractory media of the eye.

IX. On the role of monoamine oxidase in the regulation of aqueous humor dynamics of the rabbit eye

E. Albert Zeller, David Shoch, Steven G. Cooperman, and Robert I. Schnipper

To find out whether monoamine oxidase (MAO) plays any role in the dynamics of the aqueous humor, we tried to block this enzyme in the anterior part of the rabbit eye. Since trans-2-phenylcyclopropylamine, which combines the properties of a sympathomimetic amine and MAO inhibitor, appears in the anterior chamber within a few minutes after conjunctival instillation as indicated by mydriasis, it was considered probable that another aralkyl-amine, N-benzyl-N-methylpropynylamine (pargyline), one of the most potent MAO inhibitors, would reach intraocular MAO. Actual determinations with a sensitive photometric method of MAO activity in the homogenate made of iris and ciliary body confirmed this assumption. Albino rabbits, after extensive tonographic determination of their aqueous humor dynamics, received repeated instillations of the pargyline solution into the right eye. As early as 4 hours after the first instillation, we observed highly significant depression of the intraocular pressure, apparently caused by a significant reduction of the rate of aqueous formation, since no alteration in the outflow facility occurred. For two weeks of treatment the tonographic data remained essentially the same. In contrast to the phenylcyclopropylamine experiments, no change in the pupillary size took place after pargyline instillations. In the left eyes of treated rabbits and in eyes of various control animals, these changes were entirely absent. The observational data remained essentially the same for the two weeks of treatment. The mechanism of this reaction remains to be elucidated. Transfer of catecholamines or serotonin into the aqueous sufficient to produce pupillary dilatation had to be excluded as a critical event.

The autonomic nervous system and certain biogenic amines appear to play a role in the regulation of the flow of the aqueous. Since the biological effects of these amines are probably determined by their concentration and concentration gradients, their metabolism—uptake, synthesis, storage, destruction, and release—has to be known if we expect to describe the biochemical aspects of the flow system with some degree of accuracy. One of the components of the metabolic network is monoamine oxidase (monoamine:O₂ oxid-
reductase [deaminating] 1.4.3.4) which, as determined for several species, occurs with relatively high activities at the sites where the aqueous enters and leaves the interior of the eye of the rabbit and of other species. It may, therefore, exert some influence on the turnover of the aqueous humor. To block monoamine oxidase (MAO) in oculo with one of the many available MAO inhibitors could be one way to explore this problem. A careful selection of the inhibitor and its way of administration, however, are needed in order to eliminate some experimental variables. One of these variables to be avoided are changes of blood pressure which results from systemic administration of MAO inhibitors. Topical treatment, therefore, seems to be more desirable, giving us the chance to use one eye as an untreated control. After having tested several compounds in pilot experiments, we selected N-benzyl-N-methylpropynylamine (pargyline, Eutonyl; Abbott Laboratories, North Chicago, Ill.), not only because this compound is one of the most powerful of known MAO inhibitors, but because it apparently does not cause pharmacological reactions which may obscure the experimental situation.

In this paper we present our observations regarding the response of the rabbit eye to the administration of pargyline as evaluated by tonography and by measurement of MAO activity.

Materials and methods

Preparation of animals. Ten female albino rabbits of the New Zealand strain weighing between 2.5 and 3.0 kilograms were used. Since the nictitating membrane frequently interfered with the tonographic measurements, it was removed under local lidocaine (2 per cent) anesthesia. One month later tonography was carried out on all animals.

Tonography. Each rabbit was tightly wrapped in sturdy cloth to prohibit body movements and placed on its side, with support under the head so that no pressure was exerted on the opposite eye. Several minutes later, after the animal became relaxed, tonography was performed under topical anesthesia of one drop of proparacaine (Ophthaine, E. R. Squibb & Sons, New York, N. Y.) instilled into the conjunctival sac. Tonography was carried out with an electronic V. Mueller tonographer and Esterline recorder. The intraocular pressure, coefficient of outflow facility, and rate of aqueous formation were measured with modified tables based on Friedenwald’s calculations. The episcleral venous pressure was accepted as 9.0 mm. Hg. The right eye was always measured first, in the morning, and the left eye in the afternoon, at least 2 hours later in an attempt to eliminate as much as possible effects of the ophthalmitmic reflex.

Measurement of MAO activity. The activity was determined with the help of a spectrophotometric method. In this procedure, m-iodobenzylamine was converted into m-iodobenzaldehyde with a concomitant increase of optical density at 253 m/M. In a total volume of 3 ml., 0.2 ml. consisted of homogenate and 2.8 ml. of 0.36 mM. m-iodobenzylamine hydrochloride. To obtain a representative material from the anterior chamber, the iris and the ciliary body were grasped from within after transecting the globe and removing the lens, and stripped away from the ocular wall. This tissue was homogenized with the help of a Teflon pestle as previously described. Since its activity was fairly small, approximately 1 U., as compared with 29 and 2.5 U. for rabbit liver and brain, respectively, a relatively large quantity of this material was needed for a measurement, namely, 6.7 mg. per milliliter of final solution. The incubation was carried out in air at 37° C. The initial velocity, determined within 4 minutes in a Beckman DU spectrophotometer, was expressed as difference in optical density per minute and per gram tissue. One unit MAO activity corresponds to the optical difference (A) recorded under the given conditions.

Experimental design. After having secured pre-treatment tonographic values, 6 animals were randomly selected for treatment (Group I). These animals received 4 instillations of 0.002M pargyline, dissolved in the buffer, which will be mentioned, into the conjunctival sac of the right eye at hourly intervals on the first day of treatment and two instillations on all subsequent days. The solution was well tolerated by the animals. No conjunctival irritation or any other unusual response was noted. At twice this concentration, the drug did not cause discomfort in man. Furthermore, after the administration of this concentration of drug into the conjunctival sac of one of us (E.A.Z.) for several weeks, the slit lamp did not reveal any pathological changes. The left eye was not treated at all and served as a control. Tonography was performed daily, the first time 4 hours after administration of the first dose of pargyline. Two rabbits received, in
the right eye, the phosphate buffer without the inhibitor, according to the same schedule as the treated animals (Group II), and the last 2 rabbits were given neither inhibitor nor buffer (Group III), in order to determine any long-range effects of tonography alone on aqueous humor dynamics. Finally, after 14 days of treatment instillation of the inhibitor was discontinued and tonography was continued for 3 more days.

A considerable number of rabbits were studied in pilot and ancillary experiments. In this paper, however, we only report the results pertaining to 10 animals and obtained under very rigidly standardized conditions.

**Evaluation of data.** In the analysis of results, erratic tracings, and those involving mechanical difficulties were discarded. The results were calculated as follows: The average pressure (P_o), facility (C), and flow (F) were calculated for each animal prior to treatment with inhibitor or buffer, and control values were obtained for all 3 groups. The measurements of Group I pargyline-treated animals were calculated daily after treatment began, so that a time of onset of action and maximum effect could be determined. Measurements at "Day 1" refer to tonography on the day treatment was initiated. The first 3 days were taken separately, but the subsequent measurements were averaged together, since the parameters of aqueous dynamics showed little change from then on. This breakdown into individual days was not done with Groups II (buffer) or III (tonography alone).

**Results**

**Rate of diffusion of the MAO inhibitor phenylcyclopropylamine (PCP), into the eye.** When we instilled trans-2-phenyl-cyclopropylamine (tranylcypromine, Par-nate, Smith Kline & French Laboratories, Philadelphia, Pa.) into rabbit eyes, mydriasis occurred. PCP combines the properties of a sympathomimetic amine with that of a potent MAO inhibitor. This observation gave us a chance to determine the diffusion rate into the anterior chamber for an MAO inhibitor which, like pargyline, is an aralkylamine derivative. With a M/200 solution of PCP in M/15 phosphate buffer pH 7.2, instilled into the right conjunctival sac, the beginning of mydriasis took place after 4 minutes (average of 5 measurements), as compared with the pupillary size of the left eye which was treated with the buffer only. After 15 minutes, the average increase in pupillary diameter reached 1.8 mm.

**Effect of pargyline on MAO of ciliary body and iris.** Since it is well known that the quantitative response of MAO toward a given inhibitor may be markedly different when preparations are obtained from various species, we compared pargyline inhibition of liver MAO from rabbit, beef, and mouse. The pI 50-values (negative logarithm of the inhibitor concentration producing 50 per cent inhibition), after adding the inhibitor to the enzyme 5 minutes prior to the m-iodobenzylamine, were 7.1, 7.3, and 7.3, respectively. Although the rabbit enzyme may be slightly less sensitive than MAO of other species, it is still very strongly inactivated by pargyline, the latter producing 50 per cent inhibition at 0.02 μg per milliliter. We could, therefore, expect a strong inhibition of MAO of the anterior chamber, if only a small quantity of the instilled pargyline would reach the anterior and posterior chambers.

This prediction was completely fulfilled by actual determination of MAO activity of the iris and ciliary body in the untreated left and treated right eyes of 8 rabbits. The animals received pargyline according to the schedule given previously (Experimental Design) and were killed 24 hours after initiation of treatment. The tissues obtained from 2 to 3 animals were pooled together to make it possible to obtain duplicate values. For the right and left eyes, MAO activities were 0.038 ± 0.075 and 1.04 ± 0.288, respectively. For the treated eye, 7 out of 8 rate determinations were zero. Obviously, conjunctival instillations of M/500 pargyline caused efficient inhibition of MAO of iris and ciliary body.

**Effect of repeated tonography on tonographic data.** To obtain reliable base-line values for each animal before any treatment began, approximately 15 tonographic measurements were carried out during 4 weeks of the first period. We also wanted to find out whether repeated tonographic manipulations had any effect on aqueous
Table I. Tonographic data obtained during pretreatment period

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<tr>
<td>I</td>
<td>21.3 ± 0.8</td>
<td>20.0 ± 1.8</td>
<td>0.29 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>3.56 ± 0.27</td>
<td>3.03 ± 0.37</td>
<td>(15%)</td>
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<tr>
<td>II</td>
<td>21.8 ± 0.1</td>
<td>21.3 ± 1.5</td>
<td>0.30 ± 0.01</td>
<td>0.29 ± 0.00</td>
<td>4.00 ± 0.30</td>
<td>3.45 ± 0.59</td>
<td>(14%)</td>
</tr>
<tr>
<td>III</td>
<td>22.2 ± 0.2</td>
<td>21.2 ± 0.00</td>
<td>0.28 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>3.67 ± 0.10</td>
<td>3.45 ± 0.27</td>
<td>(6%)</td>
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Parentheses contain the differences between right and left eyes.

Table II. Tonographic data for treatment period

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<tr>
<td>I (Pargyline)</td>
<td>18.4 ± 1.1</td>
<td>0.29 ± 0.04</td>
<td>2.72 ± 0.46</td>
<td>18.4 ± 1.1</td>
<td>0.29 ± 0.04</td>
<td>2.72 ± 0.46</td>
<td>18.4 ± 1.1</td>
</tr>
<tr>
<td>II (Buffer)</td>
<td>21.8 ± 0.6</td>
<td>21.1 ± 1.4</td>
<td>0.30 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>3.88 ± 0.26</td>
<td>3.30 ± 0.43</td>
<td>(15%)</td>
</tr>
<tr>
<td>III</td>
<td>22.1 ± 0.1</td>
<td>21.0 ± 0.2</td>
<td>0.29 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>3.53 ± 0.25</td>
<td>3.55 ± 0.22</td>
<td>3.53 ± 0.25</td>
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Table III. Tonographic data obtained during posttreatment period (Group I)

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<th>Days after cessation</th>
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<tr>
<td>1</td>
<td>23.8 ± 0.9</td>
<td>21.2 ± 1.7</td>
<td>0.38 ± 0.03</td>
<td>0.28 ± 0.06</td>
<td>5.63 ± 0.74</td>
<td>3.46 ± 1.18</td>
<td>5.63 ± 0.74</td>
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<tr>
<td>2</td>
<td>23.6 ± 0.6</td>
<td>21.1 ± 1.3</td>
<td>0.33 ± 0.01</td>
<td>0.27 ± 0.03</td>
<td>4.81 ± 0.02</td>
<td>3.28</td>
<td>4.81 ± 0.02</td>
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<tr>
<td>3</td>
<td>22.4</td>
<td>21.0</td>
<td>0.30</td>
<td>0.28</td>
<td>4.02</td>
<td>3.36</td>
<td>4.02</td>
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Humor dynamics. The results are summarized in Table I. The data are fairly homogeneous as indicated by the standard deviations which only in a few instances go beyond 10 per cent of the means. We noticed a small difference in intraocular pressure of both eyes, possibly caused by a drop in the flow of the left eye (per cent of difference is given in parentheses, Table I). For Group I, the flow appears to be significant (P < 0.01). This was attributed to the ophthalmomotoric reflex; it did not alter the significance of our experimental observations (see the following discussion).

Effect of pargyline instillations on tonographic data. The conjunctival instillation of pargyline led to a significant reduction of intraocular pressure and aqueous flow, but not of the facility of outflow (Table II). Four hours after the initiation of the drug administration (Day 1 in Table II), the aqueous flow and intraocular pressure dropped significantly (P < 0.001). The drop both in P_o and F increased into the third day of pargyline administration. No similar changes were found in the set of data obtained from the untreated left eyes of the Group I animals during pargyline treatment nor from the right and left eyes of the rabbits receiving either phosphate buffer or no instillations at all. All statistical differences computed by comparing the P_o and F values obtained for treated (right) eyes and for untreated eyes of Groups I and II were highly significant.
The data seem to indicate that instillation of pargyline produced a drop in aqueous flow and thus a reduction of the intraocular pressure. The alterations in tonographic parameters can be maintained for at least 2 weeks although a tendency toward smaller differences during this period was apparent (fourth to fourteenth day) (Table II). At no time was a difference in the pupillary diameter of treated and untreated eyes noticed.

**Effect of pargyline withdrawal on tonographic data.** After cessation of the pargyline instillations, all tonographic data, including those for outflow facilities, for the right eye rose astonishingly fast and a day later reached values much higher than those obtained for the left eyes and for the right eyes before treatment. In the next 2 days, the figures tended to fall toward the standard values (Table III).

**Discussion**

The data presented here clearly indicate that pargyline instillations effectively, if not completely, block MAO localized in tissues surrounding the posterior chamber and presumably in other anterior parts of the eye. Evaluation of the tonographic data show a significant depression of the intraocular pressure, apparently caused by a reduction of the formation of the aqueous humor. Since these phenomena were observed under very gentle experimental conditions which did not entail the trauma of narcosis, surgery, or unusually high drug concentrations which prevail in many investigations pertaining to aqueous dynamics, they may offer new opportunities for future studies on the aqueous flow.

No receptor except MAO is known to be substantially affected by the very minute pargyline quantities applied to the rabbit eye. Not even the related enzymes, plasma amine oxidase (EC 1.5.3.3), unfortunately called monoamine oxidase in some publications, and diamine oxidase (EC 1.4.3.5) are blocked by pargyline. Preincubation of three hundred times purified beef plasma amine oxidase with 0.001M pargyline does not affect the enzyme activity as indicated by the polarographic measurement of the oxidation rate of 0.01M benzylamine. The antagonistic action of pargyline against norepinephrine in the isolated nictitating membrane requires a 10^4 times higher concentration than the inactivation of MAO. Until systems coming close to our enzyme's sensitivity are discovered, we have to assume that the local elimination of MAO is involved in the reduction of the aqueous formation. A similar situation may exist in the development of certain forms of edema which can be prevented by various types of Mao inhibitors. The destruction of MAO, however, apparently does not lead to a liberation of catechol amines in quantities sufficient to cause mydriasis. The same conclusion can be drawn for serotonin because the latter, injected into the anterior chamber, releases norepinephrine and thus induces pupillary dilatation.

Out of the vast literature, two observations may be mentioned which appear to support this conclusion: treatment of rabbits with iproniazid does not increase the norepinephrine content of the aqueous when the preganglionic cervical sympathetic nerve is stimulated; and the conjunctival instillation of epinephrine under conditions similar to those of our experiment produces an increase of outflow in human eyes, a phenomenon never observed under pargyline medication. However, we cannot discard the idea that the elevation of intracellular amine levels is responsible for the reduction of the aqueous flow.

After our tonographic work was finished, a paper appeared in which the authors showed that the intravenous injection of the MAO inhibitor nialamide caused an almost instantaneous drop of the intraocular pressure. Since this reaction runs closely parallel to the marked reduction of the blood pressure, it is difficult to separate the consequences of reduced blood pressure from that of a direct effect of the drug on ocular MAO.

At the present time, studies are under
way to find out whether local administration of MAO inhibitors is capable of affecting the intraocular pressure of glaucoma patients.

We express our gratitude to the pharmaceutical houses, the donors of the MAO inhibitors. We also want to acknowledge Dr. George M. Ruby's participation in this work and Miss Gloria J. Stanich's determination of MAO activities.

REFERENCES