Potentiation of the mydriatic effect of norepinephrine in the rabbit after monoamine oxidase inhibition. Brenda K. Colasanti and Ernst H. Barany.

Dose-response curves of pupillary dilation after topical administration of norepinephrine or methoxamine have been determined in rabbits after chronic inhibition of ocular monoamine oxidase by treatment with pargyline or pheniprazine. Eyes treated with either monoamine oxidase inhibitor showed an enhanced responsiveness to the mydriatic effect of norepinephrine given either topically or intravenously. Increments in pupil size of the treated and control eyes in response to methoxamine applied topically, on the other hand, were the same. These results suggest that monoamine oxidase may play a role in the iris as one factor influencing the concentration of norepinephrine at the receptors.

The principal mechanism for physiological inactivation of norepinephrine released from adrenergic nerve endings for action on autonomic effector organs involves reuptake into the prejunctional neuron and binding in the storage granules. Some released transmitter is also susceptible to destruction by the enzyme monoamine oxidase (MAO; EC 1.4.3.4), which is localized both within the nerve ending and extraneuronally. Because the responses of a variety of peripheral organs and tissues to directly acting catecholamines are not altered after inhibition of MAO, this route of inactivation is felt to exert little influence on the amount of transmitter available at the receptors. In contrast, MAO apparently does play an important role in determining responses to indirectly acting sympathomimetic amines, the effects of which are potentiated by MAO inhibition.

Studies undertaken with homogenates of various ocular structures have demonstrated a high level of MAO activity in the iris-ciliary body of rabbits. Recent experiments on MAO obtained from rabbit iris-ciliary body preparations 2 to 4 weeks after superior cervical ganglionectomy have indicated that this enzyme has a predominantly extraneuronal localization.

Only a limited number of studies concerning responses of the iris to drugs after MAO inhibition have been undertaken. When MAO was inhibited by topical administration of agents affecting either type A or B of the enzyme as well as those affecting both forms, pupil size of albino rabbits did not change. After topical application of the monoamine releaser Ro 4-1284 to the eyes of rabbits pretreated with these MAO inhibitors, however, pupillary dilation ensued. As in the case of responses of other peripheral tissues, the response of the iris to indirectly acting sympathomimetic agents has been shown to be potentiated in human subjects undergoing treatment with MAO inhibitors.

In the present communication, we report a potentiation of the mydriatic response of the rabbit eye to norepinephrine after prior treatment with MAO inhibitors. This increase in sensitivity of the iris contrasts with the unaltered responses of other peripheral organs and tissues to directly acting catecholamines after MAO inhibition.

Methods. Adult male albino rabbits weighing 2 to 3 kg were used in these experiments. Pheniprazine (Draco, Lund and Merrell—National Laboratories, Ohio) was applied topically by microdrop to the right eyes of three groups of six rabbits once daily for 7 days. For this purpose, a microsyringe with polyethylene 50 tubing attached to the needle was used. After proptosis of each eye, 10 μl of phosphate buffer (pH 7.4) containing 400 μg of the drug were delivered to the cornea by slow placement, over a period of 1 min, of the microdrops formed after manipulation of the micrometer. To allow the solution to dry, the eye was left proptosed for 2 additional minutes. Phosphate buffer vehicle (10 μl) was applied similarly to contralateral eyes, which served as controls. Pargyline hydrochloride (Saber Laboratories, Illinois), 2 mg dissolved in 10 μl of saline, was injected once intravitreally into the right eyes of four groups of six rabbits. An equivalent volume of saline was delivered also by a single intravitreal injection to the contralateral control eyes.

In the pheniprazine experiments, pupillary diameters in response to norepinephrine or methoxamine were determined 8 days after initiation of the chronic treatment. In the pargyline studies, pupil responses to these agonists were determined 7 days after intravitreal injection of the inhibitor. Progressively increasing doses of each sympathomimetic were applied topically to both right and left eyes at a constant volume of 25 μl. A period of 90 min intervened between successive doses. Pupillary diameter was determined every 30 min. Immediately prior to measurement, each
Fig. 1A. Changes in pupil size after topical application of norepinephrine to pargyline-treated and contralateral control eyes. Each value represents the mean ± S.E.M. for six rabbits. Asterisk, p < 0.05.

Fig. 1B. Effect of norepinephrine applied topically on the pupil size of pheniprazine-treated and contralateral control eyes. Each value represents the mean ± S.E.M. for six rabbits. Asterisk, p < 0.05.

Animal was placed on a wire net encased in a wooden frame with the eye positioned at a constant distance of 55 cm from a fluorescent light source. Pupil size was measured with a transparent millimeter ruler. All measurements were taken in the horizontal meridian and recorded to the nearest half millimeter.

In one series of experiments, pupil size of MAO inhibitor-treated and contralateral eyes was determined after intravenous administration of norepinephrine. The rabbits were placed in a commercial restrainer and the marginal ear vein was cannulated. After 10 minutes, a norepinephrine solution prepared to deliver 10 µg/kg/min of the drug was infused at a rate of 0.5 ml/min over a period of 30 min. Pupil size was measured at the termination of the infusion.

Calculations of the effective dose 50 (ED50, i.e., the dose required to produce a response 50% of the maximum) and its 95% confidence intervals for norepinephrine in the pheniprazine experiment were made in accordance with the method of Fleming et al. Because it was not always possible to obtain maximal responses to norepinephrine in
the pargyline experiment, the effective dose 1 mm (ED 1 mm; i.e., the dose required to produce an increase in pupil size of 1 mm) was calculated in a similar fashion. Statistical comparisons of all results for the treated and the contralateral control eyes were made with the use of Student's paired t test.

Results. After delivery of pargyline to the right eyes of rabbits by a single intravitreal injection, pupil size of the treated and contralateral eyes remained equal. In contrast, within 30 min after application of pheniprazine to right eyes of rabbits by the microdrop technique, all treated pupils became maximally dilated. By day 7 of the chronic daily treatment, maximal mydriasis still occurred within 15 to 30 mins after application of this MAO inhibitor. However, 24 hr later, pupil size of the treated eyes did not significantly differ from that of the contralateral controls.

After topical application of norepinephrine at progressively increasing doses, pupillary dilation of eyes treated with pargyline was significantly greater than that of the contralateral control eyes at all but the lowest doses (Fig. 1A). Calculation of the ED 1 mm for norepinephrine revealed that there was a 2.8-fold increase in sensitivity of the treated eyes over that for the controls [geometric mean (95% confidence interval) of 1.5% solution (1.3 to 1.7) for treated eyes vs. 4.3% (4.0 to 4.7) for controls; p < 0.05]. Eyes treated chronically with pheniprazine likewise showed an enhanced responsiveness to norepinephrine applied topically (Fig. 1B), with a threefold shift of the dose-response curve to the left [ED50 of 1.2% solution (1.1 to 1.3) for treated eyes vs. 3.5% (3.4 to 3.6) for controls; p < 0.05].

Changes in pupil size in response to norepinephrine given intravenously (10 μg/kg/min for 30 min) after unilateral inhibition of ocular MAO are shown in Fig. 2. Norepinephrine significantly increased the pupillary diameter of eyes treated with either pargyline (p < 0.01) or pheniprazine (p < 0.05) but not that of the contralateral control eyes. Differences in pupil size between the treated and control eyes were also highly significant (p < 0.01) in the case of both inhibitors.

After topical application of methoxamine at progressively increasing doses to eyes treated in-
travitreall with pargyline, the resulting increases in pupil size did not differ from those of the contralateral control eyes (Fig. 3A). Responses to methoxamine of eyes treated chronically with pheniprazine likewise were similar to those of the controls (Fig. 3B).

**Discussion.** The results of this study have demonstrated an enhanced responsiveness of the rabbit iris to the mydriatic effects of norepinephrine after inhibition of ocular MAO with either pargyline or pheniprazine. In contrast, responsiveness of the iris to methoxamine, a directly acting sympathomimetic which is not a substrate for MAO, remained unchanged. Because the effect of norepinephrine given intravenously was potentiated by prior treatment with pargyline or pheniprazine, changes in absorption of norepinephrine given topically as a result of MAO inhibition at ocular sites other than the iris can be ruled out. The increase in sensitivity to norepinephrine results directly from inhibition of MAO.

The mydriasis appearing shortly after application of pheniprazine to rabbit eyes is most likely due to intrinsic sympathomimetic activity in this inhibitor. As observed earlier by Zeller et al., pupil size did not change after inhibition of MAO with pargyline, a compound devoid of such intrinsic activity.

Because responses to catecholamines of the variety of peripheral tissues studied have not changed after inhibition of MAO, it has been assumed that MAO does not play a major role in the termination of the actions of norepinephrine in these tissues. The potentiation by both pargyline and pheniprazine of the mydriatic effects of norepinephrine observed in our study in rabbits indicates that in the iris MAO is capable of influencing the concentration of exogenous catecholamine at the receptors. Whether MAO also plays any appreciable role in the termination of transmitter action has to be investigated. Unlike most other smooth muscle, both the sphincter and dilator develop from neuroectoderm, and the dilator is a myoepithelium. Inhibition of MAO in the central nervous system leads to overt behavioral stimulation, presumably as a result of increased availability of catecholamines. It is possible that MAO in the iris plays a role more closely resembling that in the brain than that observed in the periphery.

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