Alterations in adrenergic sensitivity of the rabbit iris after variation of environmental lighting conditions. BRENDA K. COLASANTI AND ROBERT R. TROTTER.

After variation of the environmental lighting conditions, pupillary diameters of albino rabbits in response to progressively increasing doses of the sympathomimetics norepinephrine, epinephrine, and isoproterenol were measured. After maintenance of the rabbits under conditions of constant light for one week, an increased sensitivity of the iris dilator to the mydriatic effects of these adrenergic agents became evident. After maintenance of the rabbits in constant dark for one week, on the other hand, there was no change in the sensitivity of the iris upon administration of the same sympathomimetics. Norepinephrine levels measured in iris and ciliary processes after one week of either constant light or constant dark, moreover, did not differ significantly from those determined under normal lighting conditions. These results indicate that the sensitivity of the iris dilator can be altered by variation of the physiologic stimulus, light. In addition, the chronic interruption of contact between the normal neurotransmitter norepinephrine and its effector cells leading to supersensitivity in the dilator appears to be due to the removal of a trophic influence of norepinephrine, rather than its absolute loss.

The increased sensitivity of autonomously innervated structures to chemical mediators that follows denervation and the resulting loss of normal transmitter stores has been extensively studied in a variety of peripheral organs and tissues, including ocular structures.1, 2 In man, supersensitivity of the pupils to methacholine has been documented in patients with familial dysautonomia,3 while supersensitivity to directly acting sympathomimetics has likewise been observed in some cases of Horner's syndrome.4 Increasing the amount of neurotransmitter in the iris sphincter, on the other hand, by topical or systemic long-term treatment of experimental animals with an inhibitor of cholinesterase, has been found to cause a diminished responsiveness, or subsensitivity, of the iris to cholinergic agents.5

In a recent approach to the study of factors influencing the response of ocular tissues to drugs, Bito, Dawson, and Petrinovic6 have demonstrated that changes in the sensitivity of the iris sphincter could be produced by altering the intensity of the physiologic stimulus, light. By keeping cats in complete darkness for a period of one week, a condition which induces a temporary state of constant pupillary dilation and thereby reduces the cholinergic input to the iris sphincter, supersensitivity to the miotic effect of pilocarpine was produced. On the other hand, keeping the animals in continuous light, a situation which induces a temporary state of constant pupillary constriction and thereby increases the cholinergic input to the iris, led to the development of subsensitivity to the miotic action of pilocarpine.

In the present experiments, changes in the sensitivity of the iris dilator to sympathomimetic drugs have been examined in rabbits maintained under conditions of constant light or constant dark. In addition, norepinephrine levels of the iris and ciliary processes after these variations of the environmental lighting conditions have been determined. Our data suggest that changes in the sensitivity of the iris dilator similar to those previously reported for the iris sphincter do occur as a consequence of variation of the physiologic stimulus, light.

Methods. Adult male albino rabbits weighing 2.5 to 3.5 kilograms were used in these experimental studies. Groups of five rabbits were housed in individual cages in a room in the animal quarters which provided timer regulation of the lighting conditions. Separate groups of animals were used for the study of each drug. Upon their arrival, each group was given a period of one week to adapt to the new environment. The rabbits were then maintained for consecutive 7-day periods under the following lighting conditions: (1) 12 hours light (300 lux) and 12 hours dark every 24 hours (control); (2) constant dark; (3) a 12-hour light-dark cycle again; (4) constant light (300 lux); and (5) a final 12-hour light-dark cycle.

On the last day of maintenance at each lighting condition, pupillary diameters in response to increasing doses of each of the sympathomimetic agents studied were determined. The pupil size was measured visually with a transparent millimeter ruler. All readings were taken in the horizontal meridian and recorded to the nearest half millimeter.

Solutions of the experimental drugs were dissolved in isotonic saline and applied topically to the left eyes with a Hamilton microsyringe (Kontes Glass Co., Vineland, N. J.). Increasing
doses of norepinephrine and epinephrine were administered at a constant volume of 25 μl. Due to the limited solubility of isoproterenol, however, higher doses of this drug had to be administered in successive 50 μl volumes. A 90-minute period was allowed to elapse between successive doses of norepinephrine and epinephrine, while increasing doses of isoproterenol were given at intervals of 60 minutes.

The following drugs were used: 1-norepinephrine bitartrate (l-arterenol bitartrate); l-epinephrine bitartrate; and l-isoproterenol d-bitartrate. All three of these agents were obtained from Sigma Chemical Company, St. Louis, Mo. All concentrations were calculated as the percent solution of the free base.

Calculations of the effective dose 50 (ED50; i.e., the dose required to produce a response 50 per cent of the maximum) and its 95 per cent confidence intervals for the sympathomimetic agents under each of the various lighting conditions were made in accordance with the method of Fleming and co-workers.7 The individual values thus obtained are based on five complete dose-response curves, one for each animal. Statistical comparisons of these and all the results were made with Student’s paired t-test.

Norepinephrine levels of iris and ciliary processes were determined in separate groups of four rabbits maintained for one week under control lighting conditions, constant light, and constant dark, respectively. For the biochemical analyses, the rabbits were killed by cervical dislocation and the iris and ciliary processes were rapidly removed. The individual samples were weighed, minced with scissors, and then homogenized in a glass tissue grinder containing 5 ml. cold 0.4 N perchloric acid, 0.2 ml. 0.1M ascorbic acid, and 0.2 ml. 0.3 M ethylenediaminetetra-acetic acid (EDTA). After centrifugation of the homogenates at 9,000 g, the supernatants were filtered. The precipitates were re-extracted, and the supernatants from the two extractions were combined. Norepinephrine was separated from the samples by ion-exchange chromatography, using a Dowex 50 W-X4 resin, and was then assayed fluorometrically in accordance with the procedure of Bertler, Carlsson, and Rosengren.8 Recovery of known amounts of norepinephrine from the Dowex columns was 79 per cent.

**Results.** Under the artificial illumination afforded by the lighting conditions of the animal quarters, the pupillary diameters of rabbits maintained under control conditions consisting of a 12-hour light-dark cycle did not vary appreciably (mean ± S.E. of 5.9 ± 0.15 mm. for all 15 left eyes). Maintenance of the rabbits under conditions of constant light for seven days did not affect the pupil size measured prior to drug administration (6.1 ± 0.15 mm., p > 0.1). After maintenance of the rabbits in constant dark for one week, on the other hand, the pupillary diameters measured in the light were found to be significantly less than those seen during control conditions (4.8 ± 0.16 mm.; p < 0.05).

A graded increase in pupillary diameter of rabbits maintained under a regular 12-hour light-dark cycle was obtained in response to progressively increasing doses of epinephrine (Fig. 1) and norepinephrine (Fig. 2) applied topically at 90-minute intervals. A graded increase in pupillary diameter in response to isoproterenol under control conditions was likewise obtained after its administration at more frequent intervals of 60 minutes (Fig. 3).

Topical administration of either epinephrine, norepinephrine, or isoproterenol after maintenance of the rabbits in constant light for seven days resulted in a shift of the dose-response curves to the left (Figs. 1 through 3) and a significant
Table I. Influence of lighting conditions on the mydriatic response of the rabbit eye to some sympathomimetic drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control Constant light (7 days)</th>
<th>Constant dark (7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>2.2 (1.7 to 2.9)</td>
<td>1.1† (0.7 to 1.7)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>6.0 (4.1 to 8.9)</td>
<td>3.7† (2.1 to 5.8)</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>3.7 (3.2 to 4.3)</td>
<td>2.9† (1.7 to 3.7)</td>
</tr>
</tbody>
</table>

* n = 5.
† P < 0.05.
‡ P < 0.01.

Fig. 3. Effect of isoproterenol applied topically on the pupil size of rabbits kept in constant light or dark for one week in comparison with the control (12-hour light-dark cycle). Each value represents the mean for five rabbits.

Discussion. An increased sensitivity of the iris sphincter to cholinomimetics after lack of use induced by light deprivation, and conversely, a reduced sensitivity after constant use induced by excessive light stimulation, has previously been demonstrated by Bito, Dawson, and Petrinovic. The results of the present study further indicate that changes in the sensitivity of the iris dilator occur simultaneously, with the development of supersensitivity after excessive light stimulation. The direction of the sensitivity changes produced in both the iris sphincter and the iris dilator are in accordance with classical concepts on the phenomenon of supersensitivity in denervated tissues. Lack of use of the iris sphincter after conditions of constant dark should lower cholinergic input and, thus, effective concentrations of the neurotransmitter acetylcholine. Lack of use of the iris dilator after constant light should similarly reduce adrenergic input and effective concentrations of the neurotransmitter norepinephrine. In both cases, a supersensitivity would be expected to develop.

While supersensitivity of the iris to the mydriatic effects of the sympathomimetics was manifested in our study as a result of excessive light stimulation, a subsensitivity to the miotic actions of cholinomimetic agents was seen by Bito, Dawson, and Petrinovic in cats maintained under similar lighting conditions. These results are a reflection of the opposing physiologic roles of the iris sphincter and the iris dilator. In addition, they may provide support for the viewpoint that supersensitivity and subsensitivity are simply opposite expressions of the same phenomenon.

While supersensitivity of the iris dilator to the adrenergic agents in response to constant light developed, no change in sensitivity after con-
dations of constant dark was readily apparent. Norepinephrine levels in iris and ciliary processes after both experimental procedures, moreover, were no different from those measured under control conditions. All methods previously used for producing disuse supersensitivity share the common property of chronic interruption of the normal contact between a neurotransmitter and its effector cells. The supersensitivity observed in the iris dilator after constant light is accordingly assumed to be due to the removal of a trophic influence of the normal transmitter, norepinephrine, rather than its absolute loss. The failure for subsensitivity of the dilator to develop in response to over-stimulation by constant darkness, on the other hand, may indicate that the activity and sensitivity of this muscle is normally quite pronounced.

Supersensitivity of the iris dilator after conditions of constant light became manifest in response to the alpha agonist norepinephrine, the mixed alpha- and beta-agonist epinephrine, and the beta-agonist isoproterenol. Since pupillary dilation in response to “pure” beta-agonists is currently thought to be mediated by stimulation of alpha-receptors in the iris dilator, it seems logical to assume that a common underlying mechanism is involved in producing the observed supersensitivity to all three sympathomimetics.

Most of the studies on the phenomena of supersensitivity and subsensitivity of excitable tissues have necessarily utilized marked surgical or pharmacological intervention. The present experiments demonstrate that sensitivity changes in the iris dilator can be induced much less drastically by variation of the physiologic stimulus, light. Use of these experimental conditions should thus provide an excellent model for study of the interaction of adrenergic and cholinergic nervous systems in ocular function.

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REFERENCES


Retinal degeneration in cats fed casein. I. Taurine deficiency. SUSAN Y. SCHMIDT, ELIOT L. BERSON, AND K. C. HAYES.

All cats fed a taurine-free casein diet for at least 23 weeks have shown granularity with a hyper-reflective white zone in the area centralis, nondetectable electroretinograms (ERG’s), and structural changes indicating photoreceptor cell degeneration. The present study has demonstrated that cats fed this casein diet have a selective decrease in plasma and retinal taurine concentrations by five weeks; taurine levels were about 4 per cent of normal in plasma, and 60 per cent of normal in retina. After 10 weeks, taurine levels were 2 to 4 per cent of normal in plasma and reached a minimum of 20 to 30 per cent of normal in the retina. These biochemical changes occurred in association with a delay in the cone ERG implicit time at five weeks and reduced cone and rod ERG amplitudes at 10 weeks. During this period, retinal DNA content (as a measure of cell viability) and fundus appearance were normal. By 23 weeks, ERG’s were nondetectable, retinal DNA content was reduced, and the fundus showed typical changes in the area centralis. These studies help to establish a biological role for taurine in maintaining photoreceptor cell function and viability in the cat.