The influence of light and dark on the catecholamine content of the retina and choroid

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Recent histochemical studies with the use of a fluorescence method to detect catecholamine-containing neurons have uncovered a system of dopamine-containing amacrine cells as well as a plexus of norepinephrine-containing nerve terminals in the choroid. This study examined the effect of dark and light adaptation on the catecholamine content of the posterior segment of the eye of the albino guinea pig, rabbit, and rat by quantitative and histochemical means. In the rat and rabbit, there was a significant increase in the dopamine content with light adaptation. In the guinea pig, there was an increase, but this was not statistically significant. A significant increase in the norepinephrine content was observed in the posterior segment of the guinea pig and rabbit with light adaptation. The increase in norepinephrine appeared to be a direct effect on the choroidal nerve terminals, since with pigmented guinea pigs the increase was not observed. Likewise, decentralization of the choroid in albino guinea pigs did not alter the changes seen with light adaptation. Histochemical studies demonstrated a consistent increase in the fluorescence of the amacrine cells of the rabbit and guinea pig with light adaptation. It was not possible to show consistent changes in the rat.

A system of intraretinal dopamine-containing neurons located at the junction of the inner plexiform and inner nuclear layers of several species was described with the use of a combination of histochemical and chemical methods for identification and localization of catecholamines. In addition, a rich plexus of catecholamine-containing fibers was seen to surround the vessels of the choroid, whereas the sclera is devoid of catecholamine-containing fibers except surrounding penetrating vessels. The function of the dopamine-containing neurons is unknown. The present report compares the effect of light and dark upon the catecholamine content of the retina and choroid of the albino rat, guinea pig, and rabbit by histochemical and quantitative methods.

Methods

Only male subjects were used in this study. Albino Wistar rats, 200 to 250 grams and 11 to 12 weeks old, albino guinea pigs (Hartley strain), and pigmented guinea pigs (mongrel strains), 400 to 500 grams and 12 to 16 weeks old, were light adapted by exposure to daylight for 1 hour (illumination varied from 330 to 550 lux within the cages). Albino rabbits (New Zealand white strain), 3 to 3.3 kilograms and approximately 4 months old, were exposed to a 100 watt incandescent light bulb placed 1½ feet above each
rabbit's head for 1 hour (illumination varied from 275 to 440 lux at eye level). All the animals were in clear plastic cages without lids or bedding material during light exposure. They were constantly observed and gentle movement of the cage was used, when necessary, to assure that the eyelids remained open and the eyes exposed to light. All the animals were dark-adapted by placing them in a photographic darkroom for 24 hours. In addition, albino guinea pigs (350 to 400 grams, 10 to 12 weeks old), whose right superior cervical preganglionic nerve trunk had been cut 8 days previously, were light adapted.

Rats and guinea pigs were decapitated, and rabbits were killed by air embolism. The globes were rapidly dissected out and the anterior segment removed by cutting about 2 mm. behind the limbus. The posterior segments of guinea pig and rabbit eyes were then frozen in liquid nitrogen. The rat retina was easily separated from the underlying choroid and was frozen alone. This procedure for the light-adapted animals was performed under identical lighting conditions as those used for light adaptation. The dark-adapted animals were processed in a room adjoining the photographic darkroom and equipped with black shades. No lights were used and after an adaptation period of about 1/2 hour, the investigator could just distinguish necessary landmarks. The rabbits took longer to kill than the smaller species. Rabbits were placed in a box which was sealed so that only the ear was exposed. A second individual injected air intravenously while illuminating the ear vein with a penlight. The average dissection time, i.e., from time of death to freezing of the posterior segment, was essentially similar for the light- and dark-adapted animals; the time for rabbits and guinea pigs was about 25 seconds for the right eye and 50 seconds for the left eye. Since the retina alone was used from rats, this dissection time increased to 30 seconds for the right and 65 seconds for the left eye. Histological studies of the anterior segments from dark-adapted eyes treated in the above manner verified complete separation of the anterior from the posterior segment. All tissues were stored in a frozen state on dry ice and analyzed for norepinephrine and dopamine within 24 to 48 hours.

Each experiment consisted of two determinations, one on light-adapted and the other on dark-adapted animals. The tissue was weighed and then for each determination the posterior segments of the eyes from 3 guinea pigs or 2 rabbits or the retinas from the eyes of 3 rats were pooled. Dopamine and norepinephrine were determined spectrophotofluorometrically by the method of Hogans as quoted by Jacobowitz and associates.6 Norepinephrine was activated at 390 mλ, and the emission read at 490 mλ immediately after processing the tissue. Dopamine was activated at 330 mλ and the emission read at 380 mλ at least 20 minutes after the norepinephrine determinations. The dopamine fluorophore requires 20 minutes to develop and is then stable for several hours. (Wavelengths given are uncorrected instrumental values.)

For histochemical examination, whole eyes, light- and dark-adapted as above, were frozen in isopentane cooled in liquid nitrogen, freeze-dried in vacuo at -35° C. for 2 days and then treated with paraformaldehyde gas at 80° C. for 1 hour according to the method of Falck and associates.7-8 Paraffin sections were cut at 14 mμ and examined for monoamines with a fluorescence microscope. All photographs were developed identically and paired according to equal exposure times for comparison between the fluorescence in dark- and light-adapted animals.

### Results

Light adaptation caused an increase in the catecholamine content of the posterior segments of the eyes, as shown in Table I.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dopamine (ng./posterior segment)</th>
<th>Norepinephrine (ng./posterior segment)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>Rabbit (3) f</td>
<td>8.8</td>
<td>13.7</td>
</tr>
<tr>
<td>Rat (5) t</td>
<td>3.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Guinea pig (9)</td>
<td>3.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Guinea pig (2) pigmented denervation</td>
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<tr>
<td>Guinea pig (2) preganglionic denervation</td>
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</tbody>
</table>

*Student t test.

†Number of experiments.

‡Retina only.

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Table I. Catecholamine content of the posterior segment of the eye

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* Student t test.
† Number of experiments.
‡ Retina only.
segment of the eye in all the species studied (Table 1). The values are reported as nanograms (ng.) per posterior segment primarily because an unknown amount of vitreous was inevitably included with each specimen. This caused considerable differences between individual weights, and made it undesirable to compare values on a per gram basis. In the albino rabbit, the mean dopamine content increased from 8.8 to 13.7 ng. per posterior segment. In the albino rat, only the retina was used and there was a mean increase in dopamine from 3.3 to 5.2 ng. per retina. Although there appeared to be an increase in dopamine in the albino guinea pig from 3.5 to 4.8 ng. per posterior segment, this did not attain statistical significance (p > 0.05).

An increase in the norepinephrine of the posterior segment was found after light adaptation in the albino rabbit and guinea pig. In the rabbit, the norepinephrine increased from 16.5 to 22.7 ng. per posterior segment. In the guinea pig, there was an increase from 16.2 to 19.3 ng. per posterior segment. With pigmented guinea pigs, however, there was no comparable increase in norepinephrine with light adaptation. Furthermore, in albino guinea pigs, cutting the preganglionic trunk to the right superior cervical ganglion did not cause any difference between the two sides in the norepinephrine content of the posterior segment in response to light adaptation. The retinas of rats and guinea pigs were found to contain measurable quantities only of dopamine. Dopamine was shown previously to be the major catecholamine in the rabbit retina. The sclera is not innervated by catecholamine containing nerve fibers, although a few fibers do penetrate it in conjunction with blood vessels. Therefore, the changes in norepinephrine seen above primarily represent changes in its concentration in the choroid.

With the use of fluorescence microscopy, it was found that most of the dopamine-containing cells of the light-adapted retinas of rabbits and guinea pigs showed an increase in fluorescence intensity (Figs. 1 and 2). Also noticeable is an increase in intensity of fluorescent fibers in the inner plexiform layer of the light-adapted retinas. It was not possible to demonstrate consistent differences in the fluorescing cells of dark- and light-adapted rat eyes, probably because visual discrimination could not distinguish an increase over the already intensely fluorescent cells seen in the dark-adapted retina.

Discussion

Laboratory animals, as well as human beings, have been known to develop an
increased sensitivity to light during chronic administration of reserpine, which causes a depletion of catecholamines from nerve terminals. Malmfors, therefore, postulated that the photophobia may be due to the depletion of an inhibitory transmitter from adrenergic nerves in the retina. A possible pathway which is depleted by reserpine has been described in the rat, guinea pig, and rabbit. This pathway is considered to be intraretinal and the cells of origin have been described as amacrine cells. These cells have been shown to contain dopamine. A similar pathway arising from amacrine cells but containing acetylcholinesterase has also been demonstrated. Whether or not these two systems are identical is unknown.

The results of these experiments show that the retinal dopamine content is increased after light adaptation. This could be due to an increase in storage of the catecholamine. However, as mentioned above, reserpine causes depletion of an apparently inhibitory transmitter required for light adaptation. The increased dopamine content, therefore, may well be a manifestation of increased synthesis and release.

The values obtained for dopamine in the rabbit posterior segment are similar to those reported by Haggendal and Malmfors, compared on a nanogram per organ basis. They report that these dopamine values are equivalent to 0.1 to 0.2 μg per gram of retina, wet weight. This latter value is 10 per cent of that reported by Drujan and associates. However, he fails to state whether his values are on a wet or dry tissue basis. In addition, Drujan reported a decrease of 57 per cent in the dopamine content of the retina during light adaptation in the rabbit. This was a change from $2.202 \pm 0.610$ to $1.271 \pm 0.490 \mu g$ per gram. There is no mention of the statistical significance of this data. Likewise, the intensity of the light used is not indicated, but the time of exposure was 4 hours as compared to 1 hour in the present study. It is conceivable, therefore, that with longer exposures, or a different light intensity, a decrease in the retinal dopamine content could occur. Moreover, the length of time required to process the tissue might be important. Drujan did not indicate the dissection time. Since he removed the retina, the time for tissue manipulation was greater than that of the present study.

The enzymes, monoamine oxidase, and catechol-o-methyl transferase, responsible for metabolic degradation of catecholamines, are found in the retina and choroid. It has been shown recently that monoamine oxidase activity in the retina is increased with light adaptation. It is suggested that the increase in monoamine oxidase activity may be a reflection of
a greater demand for metabolic degradation of the increased content of retinal dopamine after light adaptation. Therefore, increased time for surgical manipulation could possibly be responsible for a decrease in the retinal dopamine content in the light adapted eye.

A surprising increase in the norepinephrine content of the posterior segment was observed with exposure to light. Since norepinephrine in the posterior segment is contained primarily in the choroid as described above, this change probably represents an increased content of norepinephrine in the choroidal nerve terminals. Two attempts were made to define the mechanism of this increase. First, in pigmented animals, most of the light would not reach the choroid, thus eliminating any direct effect of light on nerve terminals. The adrenergic nerves to the choroid emanate from the superior cervical ganglion since ganglionectomy causes depletion of catecholamines in this region. If the stimulus to an increase in norepinephrine was reflexly transmitted through the superior cervical ganglion, one would see an increase in the light-adapted pigmented animals. However, no appreciable change in the norepinephrine content was seen between the light- and dark-adapted pigmented animals, suggesting that a direct effect on the choroidal nerves may be involved. Second, with decentralization of the superior cervical ganglion prior to light adaptation, a centrally mediated reflex would affect only the intact side. The values, however, for both sides were not appreciably different. These factors suggest that light may act directly on the nerve terminals to cause an increase in choroidal norepinephrine synthesis. Similarly, increased light impinging on the eye may serve to stimulate the synthesis of dopamine within the amacrine cells of the retina.

REFERENCES