Degeneration mydriasis and hyperemia of the iris after superior cervical ganglionectomy in the rabbit

Evidence for release of more than norepinephrine during degeneration of adrenergic terminals

Giora Treister* and Ernst H. Bárány

Two intraocular phenomena following sympathetic denervation—degeneration mydriasis and degeneration hyperemia of the iris—were studied in conscious albino rabbits. The superior cervical ganglion was removed on one side (denervation) and the preganglionic sympathetic trunk was cut on the control side. Mydriasis and hyperemia were judged by comparing the two eyes. Mydriasis started about 14.5 hr. after denervation and hyperemia appeared 3 to 4.5 hr. later. Bretylium caused a markedly longer delay of the hyperemia than of the mydriasis. Neither phenoxycbenzamine, phentolamine, chlorpromazine, nor spiroperidol prevented the hyperemia, they influenced neither its starting time nor its intensity. Reserpine did not influence starting time but possibly reduced intensity. These drugs eliminated the first 1 to 3 hr. of mydriasis completely, but were only partially active on later stages. Neither propranolol nor butoxamine prevented or affected the hyperemia or the mydriasis. Systemic atropine enlarged the pupils but did not influence the hyperemia. Topical lidocaine neither prevented nor influenced the hyperemia or the mydriasis. Making the eye resistant to irritation hyperemia by repeated trauma failed to prevent the degeneration hyperemia.

Key words: cervical ganglionectomy, mydriasis, iris hyperemia, intraocular pressure decrease, norepinephrine, time factors, pharmacodynamics, sympatholytic drugs, physostigmine, bretylium, reserpine, neuroleptics, lidocaine, butoxamine.

Earlier studies have shown that the removal of the superior cervical ganglion causes three types of spontaneous transient adrenergic effects: (1) a smooth muscle contraction (rabbit iris, rabbit ear vessels, cat nictitating membrane, and iris and rat periorbital muscle); (2) a decreased intraocular pressure; and (3) a secretion from a sympathetically innervated gland (cat salivary gland). These effects were attributed...
to the leakage of norepinephrine (NE) from the degenerating nerve endings (for references see Treister and Bárány).}

With the degeneration release of NE into the iris tissue one would expect simultaneous constriction of the blood vessels, as in the rabbit ear vessels. It is puzzling, therefore, that Eakins and Eakins and Langham observed hyperemia of the rabbit iris vessels 24 hr. after denervation, when pressure decrease is marked. Hendley and Crombie found that this hyperemia was accompanied by leakage of protein into the anterior chamber. Protein in the aqueous 24 hr. after ganglionectomy, but also after preganglionic sympathotomy; had already been observed by Langham and Taylor.

Hyperemia of the iris vessels in the rabbit is present also in the well-known "intraocular irritation syndrome." Ambache found this to be associated with the presence of "irin" in the anterior chamber. "Irin" seems to be a mixture of, among other things, prostaglandins E2 and Fα. These two prostaglandins could also be released from rat stomach by sympathetic nerve stimulation and from spleen, either by sympathetic nerve stimulation or by administration of epinephrine or NE. It is not clear whether the prostaglandins are released at least partly from the nerve endings or if they derive from the surrounding tissue. Hence it is possible, that, besides NE, other substances may be released from the degenerating nerve endings or from the tissue which induce the hyperemia and leakiness of the iris vessels. These substances might also influence the mydriasis and the outflow facility and intraocular pressure.

We have studied, therefore, the pharmacological properties and time relations of the degeneration mydriasis and the accompanying hyperemia, which, for shortness, will be referred to as degeneration hyperemia. In order to distinguish the true denervation phenomena from the phenomena due to cessation of sympathetic impulse flow, the control eyes were decentralized. This had not been done in earlier work.

**Material and methods**

Albino rabbits of both sexes weighing 1.5 to 3.0 kilograms were used. Commercial food pellets and water were provided ad libitum. Left preganglionic sympathotomy (decentralization) and right cervical ganglionectomy (denervation) were performed under pentobarbital anesthesia (30 mg. per kilogram intravenously). The technique used was that employed by Sears and Bárány.

**Technique and measurement.** All the observations were made on conscious animals which were immobilized by a nylon net and placed on a wire net as described. The hyperemia of the iris vessels in the denervated eye was judged by comparing it with the decentralized eye. Five degrees of intensity were distinguished: =, both eyes equal; ≥, the denervated iris is a little redder and a slight congestion of the radial vessels and the background can be observed; >, the denervated iris is clearly redder than the control one; >>, the denervated iris shows strong congestion of vessels, slight edema of the iris tissue, and slight aqueous flare; or >>>, pink iris, maximal congestion of vessels, edema of the iris, and moderate aqueous flare.

Measurements were repeated at least hourly. The eyes were examined under ordinary light (75 W lamp) at a distance of 20 cm. from the eye during each measurement. The eyes were examined twice: first by the naked eye and then by using x2 magnification.

Several rabbits were examined by slit lamp and an attempt was made to measure the aqueous flare by a modification of Ronne's colloidometer. These measurements were not successful.

**Calculations.** The design of the calculation and the graphical representation of the mydriasis were those employed earlier. Each value represents the difference between the denervated and the decentralized pupils. As the hyperemia was measured and evaluated by a subjective method, a precise starting point could not be defined. Hence minimum and maximum points are given and one can assume that the real starting point lies somewhere in between. The minimum starting point (Tmin) is the mean between the time of the last measurement at which both eyes still were equal (=) and
the time at which the denervated eye started to be redder (≥).

The maximum starting point (Tmax) is the mean between the time at which the denervated eye showed hyperemia of ≥ degree and that where it showed > degree.

Values express means ± standard error of the means. Paired comparisons were used whenever possible and significance tested by means of the t test.

Drugs.
1. Bretylium tosylate (Dr. A. F. Green, the Wellcome Research Laboratories, Beckenham, Kent). A 1 per cent solution in 0.9 per cent sodium chloride was used.
2. Phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline and French Labs., Philadelphia). One per cent solution in acidified propylene glycol diluted with 0.9 per cent saline just before use.
3. Phenolamine methanesulfonate (Regaine ampoules, Ciba, Basel).
4. Reserpine (Serpasil ampoules, Ciba, Basel).
5. Chlorpromazine hydrochloride (Hibernal ampoules, Leo, Halsingborg).
6. Phentolamine methanesulfonate (Regitine ampoules, Ciba, Basel).
7. Reserpine sulfate, 1 per cent (ACO, Stockholm).
8. Metaraminol bitartrate, 1 per cent (Aramine, MSD, Rahway, N. J.).
9. Butoxamine hydrochloride (Burroughs Wellcome & Co., Tuckahoe, N. Y.). A 3 per cent solution in 0.9 per cent sodium chloride was used (the drug dissolved only after gentle warming of the saline).
10. Lidozaine hydrochloride, 2 per cent (Xylocaine, Astra, Södertälje).
11. Spiroperidol (Janssen Pharmaceuticals, Beerse). The drug was dissolved in 2 to 3 drops of glacial acetic acid and was diluted with 5 per cent glucose up to a 0.05 per cent solution.

Compounds number 1, 2, and 9 were kindly donated by the manufacturers. The spiroperidol was a sample lent by Dr. N. E. Andén, Gothenburg, who drew our attention to the compound.

Results

Way of expressing starting time of hyperemia. We are giving two figures, the first is Tomin, the second, Tmax. For brevity, they are shown as a range, with the lower figure indicating the average Tomin and the higher figure, the average Tmax.

The degeneration hyperemia in untreated animals. In this group of six animals, the hyperemia started 17.5 to 18.9 hr. after denervation (Table I, a, 2, 3) viz. about 3 to 4.5 hr. after the start of the degeneration mydriasis (I, a, 5, 6) (P < 0.001). The hyperemia developed relatively fast and reached its peak in 2 to 4 hr. at most (I, a, 7). Up to this time, the two irides look alike. One can see mainly the major arterial circle, and, in some subjects, very few delicate radial arteries on a bluish white background. When the hyperemia starts in the denervated eye, the radial arteries become more and more congested and visible, at first on the superior and inferior sides and then all over the iris. Later on the background of the iris becomes pink and one can see many very small congested vessels running in all directions. On the whole, the congested vessels look somewhat cyanotic. The sphincter region is by far less hyperemic.

Gradually the iris becomes edematous and a gradually increasing aqueous flare can be seen. The ciliary processes (by transillumination) become thick too. During all this the pupil continues to dilate reaching a maximum 20 to 22 hr. after denervation.

We could not observe any consistent difference between the vessels of the limbus or of the nictitating membrane of the denervated and decentralized sides. The nictitating membranes were equally hyperemic on both sides. Thus the hyperemia seemed to be strictly intraocular.

The iris of the left decentralized eye continues to be white or only very slightly hyperemic. In several decentralized eyes one can see a weak aqueous flare, but always less than in the denervated eyes.

The duration of the hyperemia was 20 to 40 hr. (n = 5).

Summary. Simple inspection 22 to 24 hr. after unilateral cervical ganglionectomy shows simultaneously three homolateral denervation phenomena: a pink, hyperemic iris with a wide pupil and a pale ear (because of the vasoconstriction of the ear vessels).

The effect of bretylium. This compound
Table I. Time relations of the degeneration mydriasis and the degeneration hyperemia (mean ± S.E.M.)

<table>
<thead>
<tr>
<th></th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Mydriasis T&lt;sub&gt;e&lt;/sub&gt; (hr.)</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Hyperemia T&lt;sub&gt;e&lt;/sub&gt; min (hr.)</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; Hyperemia T&lt;sub&gt;e&lt;/sub&gt; max (hr.)</th>
<th>Hyperemia Peak (hr.)</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; Difference 2 to 1 (hr.)</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; Difference 3 to 1 (hr.)</th>
<th>6&lt;sup&gt;th&lt;/sup&gt; Difference 4 to 2 (hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Untreated controls (n = 6)</td>
<td>14.33 ±0.66</td>
<td>17.50 ±1.23</td>
<td>18.91 ±1.05</td>
<td>20.33 ±1.04</td>
<td>3.16 ±0.80</td>
<td>4.58 ±0.45</td>
<td>3.16 ±0.33</td>
</tr>
<tr>
<td>b. Bretylium, 10 mg. per kilogram, intramuscularly at time of operation and 10 hr. later (n = 6)</td>
<td>19.90 ±1.29</td>
<td>25.40 ±0.87</td>
<td>27.60 ±1.32</td>
<td>29.10 ±1.04</td>
<td>5.50 ±0.92</td>
<td>7.70 ±0.86</td>
<td>3.70 ±0.37</td>
</tr>
<tr>
<td>c. Propranolol, 1-2 mg. per kilogram, intravenously or intramuscularly, hourly injections from 10 hr. after operation (n = 6)</td>
<td>14.41 ±0.51</td>
<td>18.00 ±0.52</td>
<td>18.92 ±0.71</td>
<td>20.91 ±0.96</td>
<td>3.58 ±0.34</td>
<td>4.50 ±0.42</td>
<td>2.91 ±0.48</td>
</tr>
<tr>
<td>d. Reserpine, 2.5 mg. per kilogram, intramuscularly 6 hr. after operation (n = 5)</td>
<td>17.25 ±0.85</td>
<td>17.62 ±0.87</td>
<td>18.62 ±0.72</td>
<td>19.37 ±1.50</td>
<td>1.75 ±1.26</td>
<td>0.25 ±0.25</td>
<td></td>
</tr>
<tr>
<td>e. Phenoxybenzamine, 10 mg. per kilogram, intravenously at time of operation (n = 6)</td>
<td>17.08 ±0.65</td>
<td>16.37 ±0.51</td>
<td>17.50 ±0.41</td>
<td>18.62 ±0.37</td>
<td>-1.25 ±0.47</td>
<td>-0.12 ±0.77</td>
<td>2.25 ±0.53</td>
</tr>
<tr>
<td>f. Phenoxybenzamine, 5 mg. per kilogram, intravenously and reserpine 1 mg. per kilogram intramuscularly at time of operation (n = 4)</td>
<td>18.33 ±0.88</td>
<td>16.33 ±1.25</td>
<td>17.44 ±1.32</td>
<td>19.16 ±0.85</td>
<td>-1.97 ±0.88</td>
<td>2.84 ±0.58</td>
<td></td>
</tr>
<tr>
<td>g. Chlorpromazine, 10 mg. per kilogram, intramuscularly every 6 hr. from the time of operation (n = 5)</td>
<td>15.83 ±0.60</td>
<td>16.52 ±0.72</td>
<td>18.00 ±0.68</td>
<td>19.00 ±0.58</td>
<td>2.17 ±0.55</td>
<td>2.48 ±0.41</td>
<td></td>
</tr>
<tr>
<td>h. Spiroperidol, 0.5 mg. per kilogram, intravenously or intraperitoneally at time of operation and every 3 to 4 hr. from 10 hr. after operation (n = 4)</td>
<td>17.12 ±0.24</td>
<td>17.25 ±0.43</td>
<td>18.25 ±0.43</td>
<td>20.12 ±0.77</td>
<td>1.12 ±0.24</td>
<td>2.87 ±0.47</td>
<td></td>
</tr>
<tr>
<td>i. Butoxamine, 30 mg. per kilogram, intraperitoneally at time of operation and every 3 to 4 hr. from 10 hr. after operation (n = 4)</td>
<td>14.66 ±0.72</td>
<td>18.33 ±0.88</td>
<td>19.00 ±1.00</td>
<td>20.53 ±0.68</td>
<td>3.06 ±0.57</td>
<td>4.33 ±0.44</td>
<td>2.20 ±0.51</td>
</tr>
<tr>
<td>j. Lidocaine 2 per cent, 4 drops every 1/4 hr. from 9 to 10 hr. after operation (n = 5)</td>
<td>14.60 ±0.62</td>
<td>17.40 ±0.55</td>
<td>18.30 ±0.70</td>
<td>19.50 ±0.63</td>
<td>2.80 ±0.60</td>
<td>3.70 ±0.73</td>
<td>2.10 ±0.19</td>
</tr>
<tr>
<td>k. Trauma + Eserine drops 0.5 per cent, (see text) (n = 4)</td>
<td>12.68 ±0.53</td>
<td>15.87 ±0.74</td>
<td>16.75 ±0.85</td>
<td>18.12 ±0.82</td>
<td>3.19 ±0.68</td>
<td>4.06 ±0.76</td>
<td>2.25 ±0.14</td>
</tr>
</tbody>
</table>

*Numbers in columns 1, 2, 3, and 4 express hours after denervation. In columns 1, 2, and 3, differences from the controls were tested; in columns 5, 6 and 7, differences from zero were tested.

| Signs in column 4 express intensity of hyperemia (see Methods). |

Paired comparisons were used for calculating the significance of differences.  

§P < 0.001; ||P < 0.01; HNot significant.
Table II. The main parameters of the degeneration mydriasis (mean ± S.E.M.)

<table>
<thead>
<tr>
<th>Drugs*</th>
<th>1 Starting point (T₀) (hr. after denervation)</th>
<th>2</th>
<th>Height (max. size) (mm.)</th>
<th>3</th>
<th>Duration (hr.)</th>
<th>4</th>
<th>Delay† (hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Untreated control (n = 6)</td>
<td>14.33</td>
<td>±0.66</td>
<td>2.58</td>
<td>±0.41</td>
<td>16.75</td>
<td>±0.83</td>
<td></td>
</tr>
<tr>
<td>b. Bretylium (n = 6)</td>
<td>19.90</td>
<td>±1.29</td>
<td>2.35†</td>
<td>±0.39</td>
<td>20.25</td>
<td>±1.01</td>
<td></td>
</tr>
<tr>
<td>c. Propranolol (n = 6)</td>
<td>14.41</td>
<td>±0.51</td>
<td>2.48†</td>
<td>±0.32</td>
<td>&gt; 14</td>
<td>0.07†</td>
<td></td>
</tr>
<tr>
<td>d. Reserpine (n = 5)</td>
<td>17.25</td>
<td>±0.85</td>
<td>1.08</td>
<td></td>
<td></td>
<td>&gt; 10</td>
<td>1.27</td>
</tr>
<tr>
<td>e. Phenoxybenzamine (n = 6)</td>
<td>17.08</td>
<td>±0.65</td>
<td>1.22</td>
<td></td>
<td></td>
<td>&gt; 10</td>
<td>2.75</td>
</tr>
<tr>
<td>f. Phenoxybenzamine + reserpine (n = 4)</td>
<td>18.33</td>
<td>±0.88</td>
<td>1.01</td>
<td></td>
<td></td>
<td>&gt; 10</td>
<td>4.00</td>
</tr>
<tr>
<td>g. Chlorpromazine (n = 5)</td>
<td>15.83</td>
<td>±0.60</td>
<td>2.18</td>
<td></td>
<td>&gt; 10</td>
<td>1.50†</td>
<td></td>
</tr>
<tr>
<td>h. Spiroperidol (n = 4)</td>
<td>17.12</td>
<td>±0.24</td>
<td>2.07</td>
<td></td>
<td>&gt; 10</td>
<td>2.79†</td>
<td></td>
</tr>
<tr>
<td>i. Butoxamine (n = 4)</td>
<td>14.66</td>
<td>±0.72</td>
<td>2.22</td>
<td></td>
<td>&gt; 14</td>
<td>0.33†</td>
<td></td>
</tr>
<tr>
<td>j. Lidocaine (n = 5)</td>
<td>14.60</td>
<td>±0.62</td>
<td>2.35</td>
<td></td>
<td>&gt; 14</td>
<td>0.27†</td>
<td></td>
</tr>
<tr>
<td>k. Trauma + eserine (n = 4)</td>
<td>12.68</td>
<td>±0.53</td>
<td>2.85</td>
<td></td>
<td>&gt; 10</td>
<td>-1.65†</td>
<td></td>
</tr>
</tbody>
</table>

*Doses of drugs are given in the text and in Table I.
†Difference (in hours) between the starting point of the untreated control group and that of the treated group.
§Not significant; @ P < 0.01; || P < 0.05.
1In column 2, differences from the controls were tested; in column 4, differences from zero were tested.

Delays the leakage of the transmitter out of the degenerating nerve terminals.\textsuperscript{5, 28-31}

In the rabbit eye it delays the degeneration mydriasis and the decrease in intraocular pressure.\textsuperscript{28} Hence it was interesting to check its effect on the degeneration hyperemia.

In one group of six animals, bretylium (10 mg. per kilogram, intramuscularly) was given at the time of operation and 10 hr. later. The hyperemia started only 25.4 to 27.6 hr. after the denervation (Table I, b, 2, 3), while mydriasis started around 20 hr. after denervation (I, b, 1). Thus, there was a larger delaying effect of bretylium on the hyperemia (7.9 to 8.7 hr., Table I, differences [b, 2] to [a, 2] and [b, 3] to [a, 3]) than on the mydriasis (5.7 hr., Table I, differences (b, 1) to (a, 1) and Table II, b, 4). Therefore, the time interval between the mydriasis and hyperemia after bretylium was increased (Table I, b, 5, 6).

The effect of propranolol. The findings of Bennett and colleagues\textsuperscript{32} (contradicted by Langham\textsuperscript{14}) indicate that vasodilator β-receptors are present in the iris vessels of the rabbit. In this group of six animals we checked the possibility that a β-adrenergic mechanism was responsible for the degeneration hyperemia by using propranolol.\textsuperscript{35, 83}

This drug's effect is of short duration in the rabbit. There is a 50 per cent reduction in its blocking potency one hour after 0.4 mg. per kilogram injected intraocularly in a conscious rabbit.\textsuperscript{32} Therefore we gave hourly injections intramuscu-
larly of a dose which is 2.5 to 5 times higher (1 to 2 mg. per kilogram) and assumed that this would maintain a full blocking effect over the whole experiment.

Tables I, a, c and II, a, c show that the time relations of the mydriasis and the hyperemia in the propranolol treated group are very similar to those of the control group. Hence, β-receptors play no role in the development of these phenomena.

The effect of reserpine. Reserpine is known to cause an almost complete depletion of the norepinephrine stores in the adrenergic nerves for several days.\textsuperscript{34, 35} This has been shown to be true also for the rabbit iris.\textsuperscript{36} If degeneration mydriasis and hyperemia are due to the release of NE, they should be prevented or markedly reduced in reserpinized animals.

Reserpine, (2.5 mg. per kilogram intramuscularly) was given at the time of operation to a group of five animals. They were kept warm with infra-red lamps. The reserpinized animals had the typical appearance: quiet, head down, complete or partial active ptosis, red swollen lids, and strained breathing. A large amount of thick yellowish secretion was present in the conjunctival sac and discharged through the nostrils. The reserpine-induced miosis\textsuperscript{37, 38} could easily be seen: 10 hr. after the injection, before the degeneration mydriasis has started, the mean pupillary size (all 10 eyes) was 3.55 ± 0.29 mm. which is well below that of the untreated group 5.0 ± 0.10 mm.

Both ears were very hyperemic during all the time of observation (11 to 40 hr. after operation). In contrast, the iris vessels on both sides were very constricted at the start of the observation period. In two animals, even the major arterial circle could not be seen. The hyperemia on the denervated side started around 17.6 to 18.6 hr. after the denervation and reached its peak 1.75 hr. later (Table I, d). While it occurred at the normal time, even at its peak the hyperemia was relatively weak, viz. $$\gg$$ or less.

Reserpine failed to eliminate the degeneration mydriasis, but the starting point of the mydriasis was delayed in all animals by an average of 2.9 hr. (Table II, d, 4). In one animal, the mydriasis was very small (0.7 mm.) and of very short duration (3 to 4 hr.). In the other four animals the mydriasis reached an average height of 1.1 mm. (Table II, d, 2), which is smaller than that of the control group ($P < 0.05$). Its duration was between 10 and 18 hr. Table I, d, 5, 6 shows, however, that the starting times of the mydriasis and the hyperemia are much closer together than in the controls (I, a, 5, 6).

An attempt was made to test if reserpine itself can cause the hyperemia by releasing the responsible substance from the degenerating nerve endings. The drug (2.5 mg.) was given intramuscularly to a group of four rabbits. The superior cervical ganglion on one side had been removed 4 days before the injection making the eye free of sympathetic innervation;\textsuperscript{52} the other side was intact. Around 30 minutes after the injection both eyes became miotic. There was no difference between the iris vessels of the two sides during a period up to 8 hr. from the injection. In two animals, both eyes were white and in two, the irides were slightly but equally hyperemic.

The effect of phenoxybenzamine. In this group of eight animals we tested the possibility that α-receptors somehow are involved in the hyperemia. Phenoxybenzamine (10 mg. per kilogram), an irreversible α-blocker, was given intravenously (using the decentralized ear) at the time of operation. The injection was given early so as to avoid its sympathomimetic effect\textsuperscript{10} which appears in the first several hours after the injection.

The drug has a very long action so that it could be given early (at time of operation). However, in order to be sure that the block was complete during the whole experiment we made several extra checks: (1) We gave Aramine (0.05 ml.) at 1 mg. per milliliter intracutaneously in the ear at
the end of the experiment and observed if there was any local vasoconstriction. (2) In two animals, we gave a second injection of phenoxybenzamine 10 hr. after the operation. (3) We observed the ear for a possible vasoconstriction.

In all the animals, both ears were very hyperemic during the whole experiment (an untreated denervated ear shows vasoconstriction around 20 hr. after the operation). Aramine did not cause any change in the diameter of the vessels, and the two animals which were injected twice with phenoxybenzamine had similar starting times for the mydriasis and the hyperemia as the other four animals.

After phenoxybenzamine, both irides became very hyperemic in some animals and it was more difficult than usual to observe differences in hyperemia between the denervated and the decentralized eye. In two out of eight animals no difference between the two sides could be observed. In the other six animals, the hyperemia appeared at the same time, or even somewhat earlier than in the control group (Table I, a, e, 2, 3).

Surprisingly enough, phenoxybenzamine did not eliminate the degeneration mydriasis completely.

In two animals, an abortive type of mydriasis started 15 and 16 hr. after the operation, continued for about four hr. and reached 0.4 and 0.6 mm., respectively. These are the animals which also did not show any difference in hyperemia.

In the other six animals, the mydriasis started on an average of 17 hr. after the operation (Table I, e, 1 and II, e, 1). Compared to the starting time of the control group, this is a delay of 2.75 hr. (Table II, e, 4). The height of the mydriasis was 1.22 mm., which is smaller than that of the control group (2.58 mm.) (Table II, e, 2). The duration of the mydriasis was between 10 and 18 hr.

Table I also demonstrates that the interval of 3 to 4.5 hr. which separated the onsets of the mydriasis and the hyperemia in the control group had disappeared in the phenoxybenzamine group (Table I, e). Both phenomena appeared quite close to each other.

In three animals which had been treated with phenoxybenzamine at the time of operation, we injected phentolamine (5 mg. per kilogram), a reversible α-receptor blocker, intravenously. The injection was given around 20 hr. after the denervation when the hyperemia had reached its peak. There was neither any change in the hyperemia nor, to our surprise, in the mydriasis.

The effect of combined phenoxybenzamine and reserpine. In order to compensate for the possibility of either incomplete block by phenoxybenzamine or incomplete NE depletion by reserpine we gave a group of four animals a combined treatment of phenoxybenzamine (5 mg. per kilogram intravenously) and reserpine (1 mg. per kilogram intramuscularly) at the time of operation. All the animals showed the reserpine syndrome.

Table I, f shows that the starting time and the development of the hyperemia in this group were not significantly different from those of the phenoxybenzamine group and the reserpine group. The delay of the mydriasis in this group was somewhat, but not significantly (P > 0.05), larger than those of the single-drug group.

The effect of phentolamine. The results of the previous experiments with reserpine and/or phenoxybenzamine point to a possibility that the first 2 to 3 hr. of the degeneration mydriasis are purely induced by NE release, while the rest of the mydriasis is the result of a combined action of NE and another unidentified substance (see Discussion). If this is true, then phentolamine, a short acting reversible α-blocker, given within the first 2 to 3 hr. of the mydriasis must eliminate it completely; given later (around 17 hr. after the denervation) it should cause only partial reduction of the mydriasis.

Table III presents results which corroborate the hypothesis. It was rather difficult to define the mydriasis at its very
Table III. The effect of repeated injections of phentolamine on the degeneration mydriasis and hyperemia (n = 4)

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* = Numbers represent the difference between the denervated and the decentralized pupils in millimeter.
† = The upper number represents the pupillary difference before injection; the middle number represents the dose of phentolamine in milligrams per kilogram given intravenously; the lower number represents the pupillary difference measured 15 minutes after the injection.
1 = Signs express the degree of intensity of the hyperemia (see methods).
onset because of its small size (around 0.5 mm.) and because it can easily be covered by the light reflex.

However, in the first two animals such a difference of 0.5 to 0.7 mm. between the pupils could be definitely measured 1 hr. after the start of the mydriasis. Phentolamine (1 mg. per kilogram intravenously) eliminated the early mydriasis completely (Table III, Exp. 1, 2).

In Exp. 2 (Table III) phentolamine (1 mg. per kilogram) eliminated the mydriasis completely even 3 hr. after its start, while in the other three animals, the mydriasis at this time was only reduced. Doubling the dose did not change the magnitude of the drug effect, only its duration.

The next dose was given 7 to 9 hr. after the start of the mydriasis, viz., within the apex plateau of the phenomenon or just at the start of its descending limb. At this stage, one could expect a maximal rate of NE release, therefore we gave more phentolamine (5 mg. per kilogram).

Table III shows that even such a high dose could only reduce, but not eliminate, the mydriasis. In animal 3, another injection which was given 10 hr. after the start of the mydriasis (3 hr. after the previous injection of 5 mg. per kilogram) did not change the already reduced mydriasis.

The maximal size of the mydriasis under the influence of the phentolamine was between 0.9 to 1.1, very similar to that seen under the influence of phenoxybenzamine and/or reserpine.

The fact mentioned above—that phentolamine given to already phenoxybenzamine-treated animals, 20 hr. after the denervation, did not reduce the mydriasis—also fits into the picture.

The hyperemia was not influenced at all by the phentolamine treatment. Table III shows that a correlation exists between the development (ascending part) of the hyperemia and the phentolamine-insensitive mydriasis. If there was hyperemia, the mydriasis was not completely reversible.

**Dopamine blocking agents.** Dopamine causes renal and mesenteric vasodilatation, by stimulating a special vasodilator dopamine receptor (for references see Yeh, McNay, and Goldberg).

Since it was not completely inconceivable that the hyperemia could be due to release of dopamine on a system containing a vasodilator dopamine receptor, we tried to block the hyperemia with dopamine blocking agents.

The neuroleptic drugs chlorpromazine, haloperidol, and spiroperidol block both dopamine and α-adrenergic receptors in the central nervous system. Haloperidol and especially spiroperidol have a marked selectivity for dopamine. The selectivity of these drugs for the peripheral dopamine vasodilator receptor is not known; this receptor is efficiently blocked by haloperidol. In the hope that the high specificity of spiroperidol would also be true for the peripheral receptor, we chose to test chlorpromazine and spiroperidol on the hyperemia.

**The effect of chlorpromazine.** The drug (10 mg. per kilogram) was given intramuscularly to a group of five animals every 5 hr. (The duration of a full effect at 5 mg. per kilogram is about 6 hr. in the rat.)

The rabbits were covered with an electric pillow to prevent a decrease in body temperature. The animals were quiet and showed slight ptosis and moderate miosis while these still were equal. The mean pupillary size was 4.0 ± 0.12 mm. compared to 5.0 ± 0.10 mm. in the control group.

The onset of the hyperemia was 16.5 to 18 hr. after the denervation, which is somewhat early but not significantly different from the control group (Table I, a, g, 2, 3).

The mydriasis started 15.83 hr. after denervation, 1.5 hr. later than the control group. However, this difference is not significant (Table II, g, 4). The height of the mydriasis was 0.4 mm. smaller (n.s.) than that of the control group (Table II, g, 2). The duration of the mydriasis was between 10 and 18 hr. One animal showed
an abortive type of mydriasis: it started 15 hr. after the operation, reached a maximum of 1.0 mm. and ended after 5 hr. The hyperemia in this animal appeared at the normal time for the group and was of normal intensity.

Comparing the starting times of the mydriasis and the hyperemia gave variable results: if the $T_{\text{omin}}$ value of the hyperemia is used one finds that both phenomena appeared almost at the same time (Table I, g, 5); if the $T_{\text{omax}}$ value (Table I, g, 6) is used one finds a later ($P < 0.01$) appearance of the hyperemia. Thus, perhaps the $\alpha$-block caused by chlorpromazine was not complete.

**The effect of spiroperidol.** We gave repeated doses of spiroperidol (0.5 mg. per kilogram intravenously or intraperitoneally). The first dose was given at the time of operation and the subsequent doses every 3 to 4 hr. from 10 hr. after the operation. Similarly to reserpine and chlorpromazine this drug caused miosis and active ptosis in both eyes. The frequency of injections was guided by the decline of the drug-induced miosis.

Four animals were used. The hyperemia in the denervated eye developed almost identically to that in the control group (Table I, a, h, 2, 3). The mydriasis, however, was significantly delayed (Table II, h, 4). The size of the mydriasis was somewhat, but not significantly, smaller than that of the control group (Table II, h). The duration was between 10 and 18 hr. The starting time of the mydriasis ($T_\alpha$) was very similar to the $T_{\text{omin}}$ of the hyperemia, but about 1 hr. earlier than the $T_{\text{omax}}$ of the latter (Table I, h, 5, 6). Thus, there seems to have been an incomplete $\alpha$-block and no special effect on the hyperemia.

**The effect of butoxamine and atropine.** In order to find out if the hyperemia was due to a metabolic effect of the released catecholamine, butoxamine, which is a selective inhibitor of this effect, was used. This drug blocks the epinephrine induced rise of plasma free fatty acid (FFA) and blood glucose. In contrast to previous metabolic antagonist, butoxamine has neither a $\beta$-blocking effect nor an $\alpha$-adrenergic stimulating one. In the dog, doses as low as 1.0 mg. per kilogram block the rise of plasma FFA induced by epinephrine. Five hours after butoxamine (15 mg. per kilogram) the block is still complete. Data concerning the duration of the butoxamine effect in rabbit were not available. In the rat a single injection of butoxamine (25 mg. per kilogram intraperitoneally) caused a marked lowering of serum FFA levels.

In the present experiment the drug (30 mg. per kilogram) was given intraperitoneally at time of operation and every 3 to 4 hr. from 10 hr. after the operation. Tables I, i, and II, i, show that butoxamine influenced neither the hyperemia nor the mydriasis, i.e., there was no significant difference from the control group.

Twenty-four hours after the operation, when both the hyperemia and the mydriasis in the denervated eye had reached their peaks, atropine (1 mg. per kilogram) was given intravenously. The atropine caused dilatation of both pupils, however, a difference of 0.5 to 1.0 mm. still remained between the eyes. There was no influence on the hyperemia in the denervated eye.

**Effect of lidocaine.** Sears has shown that lidocaine, given either as drops or as retrobulbar injection, can prevent the appearance of the irritation phenomenon (see Introduction) and the hyperemia which is one of its main signs. From this and other observations it has been concluded that the irritation phenomenon depends on an axon reflex. Considering this, it was important to see if the degeneration hyperemia too can be prevented by similar treatment. Therefore, we gave a group of five animals four drops of lidocaine (2 per cent without vasoconstrictor) every 30 min. from 9 to 10 hr. after the operation. Tables I, j, and II, j, show that the local anesthetic influenced neither the hyperemia nor the mydriasis which appeared as in the control group.
The effect of desensitization of the eye to irritation. The rabbit eye has been shown to develop resistance to repeated irritation.\textsuperscript{17, 18} The intensity of the ocular response to the irritation gradually disappears. As we wanted to avoid the use of intraocular techniques like scratching of the iris, and, since scratching of the corneal epithelium did not give the expected irritative reaction, we tried to produce a slight concussion of the right eye.

Three to four flicks of the finger caused miosis and strong hyperemia of the iris vessels, which looked similar to the degeneration hyperemia. The procedure was repeated every day or every other day five times, disappointingly without any change in the intensity of the reaction.

Eserine drops (0.5 per cent) had been found by Larsson\textsuperscript{17} to cause irritation and hyperemia of the iris vessels; this irritation hyperemia could not be produced after several applications of the drug.

The same four animals that were finger-flicked before were used for the eserine experiment. Each eye received 3 to 4 drops of eserine (0.5 per cent) every day. The hyperemic reaction differed considerably from the degeneration hyperemia. It was, on the whole, a slight reaction that started from the sphincter region (there was, of course, miosis with 1.5 to 2 mm. pupil) and showed segments of dilated radial arteries. In some animals it extended toward the periphery of the iris while in others it was restricted to the sphincter region. The color of the dilated vessels was bright red and not the purple color of the degeneration hyperemia. The resistance to the eserine-induced irritation developed relatively fast, after 2 to 3 applications. Tables I, k, and II, k, show that the repeated finger flicking and the resistance to eserine-induced hyperemia influenced neither the degeneration mydriasis nor the hyperemia.

Discussion

The degeneration hyperemia of the iris vessels is a transient phenomenon after cervical ganglionectomy in the rabbit. It is evidently not due simply to loss of sympathetic tone, since this loss is the same on the decentralized control side.

The degeneration hyperemia is surprisingly resistant to pharmacological agents:

1. Atropine had no influence on the hyperemia while causing a bilateral strong mydriasis.

2. The hyperemia was not prevented nor influenced at all by any of the following \(\alpha\)-adrenergic blocking agents: phenoxylbenzamine, phentolamine, chlorpromazine, or spiroperidol. Hence, one can conclude that it is not directly mediated by \(\alpha\)-adrenergic receptors.

3. The hyperemia could not be prevented by pretreatment with reserpine. It is very reasonable therefore to believe that it is not caused by the released NE or dopamine. The intensity of the hyperemia however, probably was weaker in this group.

4. The hyperemia was not influenced by chlorpromazine or spiroperidol. The latter, especially, is a strong blocker of central dopamine receptors, which seem to be pharmacologically similar to the vasodilating dopamine receptors. Hence, it cannot be mediated by such a dopamine receptor.

5. The hyperemia cannot be mediated by \(\beta\)-receptors, as it was not influenced by propranolol.

6. The hyperemia probably is not due to stimulation of the "metabolic receptor" for catecholamines, since it was not influenced by butoxamine. However, the duration of the effect of this drug is not well studied in the rabbit. Fortunately, both \(\beta\)- and \(\alpha\)-adrenergic blocking agents are also known to block the catecholamine-induced release of FFA\textsuperscript{51} and did not influence the hyperemia.

7. The hyperemia could be delayed by bretylium, and it was delayed considerably more than the mydriasis.

Considering all this information it is clear that the hyperemia cannot be due to a muscarinic action. Nor is it directly
due to released NE or dopamine. It is improbable that it is even indirectly due to released NE, since it is delayed more by bretylium than is the start of the degeneration mydriasis, which undoubtedly is due to released NE.

The hyperemia cannot very well be due to a lack of NE since it starts during a phase of excess release of NE and has a limited duration.

Most probably the hyperemia is due to a "substance X" released from the degenerating nerve endings, differing from NE and released somewhat later.

The hyperemia was possibly weakened by pretreatment with reserpine. This effect ought to be verified by a more quantitative method than our subjective grading of the difference between the eyes. If the effect of reserpine is real, it could have several explanations. One, that reserpine causes premature release and depletion of "substance X" is unlikely since we found no hyperemia in intact eyes in the hours immediately following a reserpine injection.

The degeneration mydriasis starts about 14.5 hr. after the denervation and continues for about 18 hr. In a previous paper we attributed the mydriasis to a leakage of NE out of the degenerating nerve endings in the dilatator region. Therefore, it was surprising to find that the mydriasis, except for its early part, shares many properties with the hyperemia. The fact that the mydriasis could not be completely eliminated by phenoxybenzamine, phentolamine, and/or reserpine indicates that NE is not the only substance which is responsible for the mydriasis. After pretreatment with phenoxybenzamine and/or reserpine, the mydriasis was delayed and started close to the start of the hyperemia, viz., 3 to 5 hr. after the normal mydriasis. This delayed mydriasis had a smaller size. It seems, therefore, that NE is exclusively responsible for only the first few hours of the mydriasis. Then, around 17 hr. after the denervation, a new factor, "substance X," appears, causing the hyperemia and contributing also to the already ongoing mydriasis. Accordingly, during the first short period, the mydriasis is fully reversible by phentolamine while during the second period it is partially resistant to the drug. It is very probable that in an untreated control group the height (maximal size) of the mydriasis is the result of a combined action of NE and the X substance.

In our previous paper we discussed the fact that the wave of degeneration mydriasis comes later than would be expected from the NE depletion of the iris reported by others. We attributed this disagreement to the gradually developing denervation supersensitivity of the iris and to accumulation of transmitter in the anterior chamber. We now believe that a third cause of the time lag is the fact that two different substances are responsible for the degeneration mydriasis, one coming into action a few hours later than the first, which is NE.

The third transient intraocular phenomenon following ganglionectomy is the increase in outflow facility and the decrease in intraocular pressure. The facility aspect of the effect is the one that has been most closely studied. In the present connection, however, we could only study pressure as very prolonged observation was necessary. An effect on facility would show up in pressure but there are other determinants of pressure and we realize that the ganglionectomy effect on pressure could be a complex one.

Simultaneous measurements of the mydriasis and the pressure decrease in the same animals have shown that the pressure decrease comes about 4 hr. later than the mydriasis. If the sympathetic nerves of the iris are the common source of the NE for both the mydriasis and the pressure decrease, this time lag poses a problem. We have shown that neither denervation supersensitivity nor reasonable values of rate of turnover of the aqueous could explain the time lag. Moreover, and most importantly, administration of bretylium delayed the pressure decrease much more than the mydriasis and increased the time...
difference between the two to 9 hr.\textsuperscript{28}

Thus, there is a remarkable similarity between the pressure decrease and the hyperemia: their starting times are quite close and bretylium delays both of them to much the same extent and considerably more than it delays the mydriasis.

Hence, it seems probable that the pressure decrease after denervation ("the ganglionectomy effect on pressure") is caused at least partly by "substance X." If this is true also of the ganglionectomy, effect on facility is not clear.

Sears and his collaborators\textsuperscript{30} have provided much suggestive evidence for the view that the ganglionectomy effect on facility is in fact due to released NE. There are, however, puzzling facts which indicate that NE may not be the only agent responsible. Reserpine does not completely prevent the facility effect\textsuperscript{10,19,39} and the effect is remarkably hard to block completely by a variety of drugs affecting the adrenergic system.\textsuperscript{5,10,15,53} Since we have made no direct experiments we cannot claim that "substance X" is involved in the facility effect, but it should be mentioned that Hendley and Crombie\textsuperscript{15} have suggested already that the facility effect is caused by something more than the release of NE.

There is a variety of vasoactive substances which could conceivably be released when nerve terminals degenerate, for instance, prostaglandins, adenosine derivatives, histamine, kinins, and other polypeptides. We have no clue as to which group "substance X" belongs to.

**Addendum**

The experiments described above were made during the winter 1969/70. In the spring of 1970 the department moved to new quarters and the animal house routine was changed. When Drs. Bill and Wilke tried to reproduce and extend the experiments during the early summer of 1970 they failed to observe as marked and consistent denervation effects as described herein. The paper was withdrawn, therefore, pending further investigation. In August, 1970, however, marked and consistent denervation changes reappeared.

There have been no conscious changes in the composition of the diet of the animals, but they are freshly bought from various breeders using different rations and a dietary factor may be at work. Such a factor might be the reason why degeneration mydriasis has not been consistently observed by earlier workers.

**REFERENCES**


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