Mydriasis and intraocular pressure decrease in the conscious rabbit after unilateral superior cervical ganglionectomy

Giora Treister* and Ernst H. Bárány**

Hourly measurements of the transient mydriasis and intraocular pressure decrease after right cervical ganglionectomy and left cervical sympathectomy were simultaneously performed in conscious rabbits. The times of onset of these phenomena after the denervation were 14.51 ± 0.21 hr. (n = 8) and 18.71 ± 0.78 hr. (n = 7), respectively. Their duration values were 18.01 ± 0.89 hr. (n = 8) and 18.81 ± 1.02 hr. (n = 4). The magnitudes of the mydriasis and the pressure decrease were 2.78 ± 0.26 and 6.00 ± 0.51 mm. Hg, respectively. The degeneration mydriasis has its peak 2 to 3 hr. later than one could expect from published data on the postdenervation transmitter disappearance from the iris. This time lag could be caused by denervation supersensitivity. Degeneration mydriasis is not affected by bilateral adrenalectomy or hexamethonium. A residual mydriasis was found to persist after the degeneration mydriasis had subsided and was also unaffected by hexamethonium or bilateral adrenalectomy. Probably it is due mainly to the transmitter remaining in the aqueous humor. The pressure decrease was found to occur 4 to 5 hr. later than the mydriasis and 1 to 3 hr. later than the appearance of tritiated norepinephrine in the anterior chamber reported by others.

Key words: cervical ganglionectomy, sympathectomy, mydriasis, intraocular pressure decrease, pupil diameter, hexamethonium, adrenalectomy, iris dilator muscle, Mackay-Marg tonometer, rabbit.

The removal of the superior cervical ganglion causes three different types of transient spontaneous adrenergic effects: (1) a contraction of sympathetically innervated smooth muscle, which was called by Langer1 “degeneration contraction” (this effect was studied in the cat nictitating membrane1 and in the rat periorbital smooth muscle3); (2) a secretion from the sympathetically innervated gland which was studied in the salivary glands by Coats and Emmelin2 and was called “degeneration secretion” as an analogue to the similar phenomenon observed in the parasympathetically denervated glands; and (3) a decrease in intraocular pressure (IOP)
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in the rabbit, which was first observed by Linner and Prijot \(^5\) and has been called \(^6\) “the ganglionectomy effect.”

It is now well established by pharmacological, \(^1, 3, 6, 7\) biochemical, \(^5, 9\) fluorometric, \(^10, 11\) histochemical, \(^13, 14\) and electron-microscopical methods \(^14, 15\) that these denervation phenomena are caused by leakage of stored transmitter from degenerating nerve endings. Surprisingly enough, the expected denervation contraction of the sympathetically innervated dilator muscle of the iris, the degeneration mydriasis, has been claimed not to occur at all \(^1, 15\) or for a very short time (1 to 2 hr.) \(^10\) and in an inconsistent manner. \(^17\)

The third denervation phenomenon, namely the IOP decrease, was found to occur 20 to 24 hours after the ganglionectomy and to be mainly due to an increase in the outflow facility. \(^6, 7, 10, 15\)

Bárány\(^6\) experiments pointed to the iris sympathetic nerve endings as the main source of transmitter. Reaching the outflow channels via the aqueous humor, the transmitter was thought to increase outflow facility and decrease IOP. Evidence and references concerning this hypothesis are summarized in Rosser and Sears\(^19\) report.

While the relationship between the iris and the IOP decrease seems to be indirectly established, no detailed study concerning the existence or nonexistence of the degeneration mydriasis has been performed, nor has the time course of this effect and the IOP decrease effect been determined. The present paper studies the time course and magnitude of these intraocular denervation phenomena in the conscious rabbit. It indicates that a typical degeneration contraction of the dilator muscle of the iris does occur and that a 4 hour interval separates it from the start of the decrease in the IOP.

Methods and materials

Experimental animals. Adult pigmented rabbits of both sexes, weighing 1.7 to 3.5 kilograms, were used. Commercial food pellets and water were provided ad lib.

Surgical procedure. Left preganglionic sympathectomy (decentralization) and right cervical ganglionectomy (derervation) were performed under pentobarbital anesthesia, 30 to 40 mg. per kilogram, intraperitoneally, adding ether as required. The technique used was that employed by Sears and Bárány. \(^7\) The operations were done mostly in the afternoon between 5 and 7 p.m. Three rabbits underwent bilateral adrenalectomy 24 hours prior to decentralization and denervation. The technique used was that of Zak and associates. \(^20\) These animals were given 25 mg. of cortisone acetate and 5 mg. of deoxycorticosterone acetate intramuscularly at the time of operation. They were kept on one per cent NaCl in the drinking water.

Drugs. All the drugs were dissolved in 0.9 per cent NaCl and given intramuscularly, except where otherwise stated. Doses refer to salt. The following drugs were used: Biperiden HCl at 2.5 mg. per milliliter and hexamethonium bromide at 20 mg. per milliliter.

Technique of measurement. All the observations were made on conscious animals, handled with care so as not to irritate them.

Each rabbit was enveloped in a nylon net, with only the head free to move, and it was placed in its natural crouching position on a wire net (Fig. 1, A and B). The nylon net which enveloped the rabbit was fixed to the wire net by snap hooks. This arrangement enabled us to hold the rabbit in place for 48 to 52 hours, with free discharge of the excretions. Food and water were provided as usual during this period.

At the time of measurement, the rabbit on its wire net was placed on a rotatable platform (Fig. 1, C). This arrangement avoided struggling and enabled us to keep the rabbit at a constant distance from the light source.

Measurement of pupillary size. The lens was caused to fluoresce by an ultraviolet light source (Fig. 1, D), a LUMA N6 HgU, 80 W ultraviolet lamp encased in a lamp house. The distance between the eye and the light source was kept constant at 40 cm. An angle of about 45 degrees between the axis of the eye and the light source prevented the shadow of the spring bow caliper from falling upon the pupillary margins. The fluorescence of the lens was sufficient to cause a moderate light reflex.

The horizontal diameter of the pupil was measured by a spring-bow caliper with fluorescent tips. (The tips of the caliper had been put into rubber latex, and after the rubber film had dried, the tips had been dipped in one per cent fluorescein sodium which stained the film. In the dark room the fluorescent tips could be clearly seen under the ultraviolet illumination.) The tips of the caliper were held very close to the cornea and
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Fig. 1A. Diagram of experimental arrangement, showing wire net on wooden frame. Dimensions are given in centimeters.

Fig. 1B. Rabbit enveloped in a nylon net connected to the wire net by snap hooks.

Fig. 1C. Rotatable platform.

Fig. 1D. Ultraviolet lamp.

about 30 cm. from the observed eye. Then the tips were adjusted precisely to the border between the dark edge of the iris and the fluorescence of the lens. The distance between the tips was measured with a ruler with interpolation to 0.1 mm. Measurements were repeated at least hourly, except for 4 hours of night sleep (for the experimenter) which were taken during non-crucial periods. Each pupillary size value was determined as the calculated mean of at least 4 successive single readings. The single readings varied at most by ±0.1 mm.

Measurement of intraocular pressure. The IOP was measured by a Mackay-Marg electronic tonometer under topical anesthesia with benoxinate (Novesine), 0.4 per cent or lidocaine, 2 per cent. After many successive applications of the tonometer probe, one gets corneal abrasions. The uneven cornea disturbs further measurements. Changing from lidocaine to Novesine made no difference. In order to prevent or to decrease this complication, we performed hourly IOP measurements only during crucial periods, which had been determined by pilot experiments. During the rest of the experiment, the IOP was measured at 2 to 3 hour intervals. According to the operating instructions, 10 to 15 quick successive applications were performed on each eye at each occasion. The lowest consistent value was used.

However, it was found to be rather difficult to determine small pressure changes (≤ 2 mm. Hg) occurring during the experiment. A slow gradual decrease in IOP was observed in several rabbits after immobilization for 30 to 50 hours. The decrease of about 4 mm. Hg was equal in both eyes, the denervated one and the decentralized one. As we were interested mainly in the pressure difference between the eyes, this phenomenon did not interfere unduly. Nor was it essential to check that the absolute calibration of the instrument holds for the eyes of our rabbits.

Calculations. Each value of the degeneration mydriasis represents the difference (D) between the means of at least 4 successive readings of the denervated and the decentralized pupils. Individual curves were constructed by plotting D-values against time after denervation. Then, the time for predetermined percentages of peak effect were
Fig. 2. Graphic representation of the main parameters of the degeneration mydriasis and intraocular pressure decrease.

read by interpolation. These were then averaged for corresponding percentages on the ascending or descending limb of the curve. Each individual and mean curve was analyzed from the following points of view (Fig. 2): (1) the time course of the effect; (a) the duration, which was measured as the difference between the onset (T_o) and the end of the effect (T_e); (b) the width, which was measured as the difference between T_50a and T_50d. (2) the height—the magnitude of the effect—the peak difference between the pupils; (3) the slopes of the limbs. The differences between T_50a and T_50d, or T_50d and T_75d, estimated on the individual curves are an expression for the inverse slope of the ascending and descending limbs, respectively. The higher the slope, the lower the value.

The pressure effect was studied in the same way as described above. Each value represents the IOP difference between the decentralized and the denervated eye except where otherwise stated.

**Results**

**Terms used.** The degeneration mydriasis, or the mydriatic effect, are different expressions for the degeneration contraction of the dilator muscle of the iris after cervical ganglionectomy. The pressure effect, or the pressure decrease effect, are different expressions for the decrease in IOP after cervical ganglionectomy.

**Light reflex.** During the starting and final periods of the denervation mydriasis, when the differences between the pupils were rather small, we used to perform two sets of measurements without and with cycloplegic. As atropine is hydrolyzed very fast in some rabbits we used biperiden 0.75 mg. per kilogram which gives an atropine-like action of longer duration, with the dosage used, 2 to 4 hours. The drug was given systemically in order to assure equal distribution. The effect of atropine or biperiden was found to be quite complex. When given prior to the start of the denervation contraction, both of the pupils were dilated to the same size. When the drug was given during the denervation contraction, the larger, denervated pupil increased as much as or, more often, more than the decentralized one. When the drug was given, however, at or after the end of the degeneration contraction the difference between the pupils either became smaller or did not change with application of the drug.

**Residual tone of the dilator pupillae muscle.** At the end of the degeneration mydriasis, the denervated pupil in the
Fig. 3. Individual curve of the degeneration mydriasis demonstrating the hexamethonium-resistant residual tone. Hexamethonium bromide 10 mg. per kilogram.

Fig. 4. Mean curves of the degeneration mydriasis and the intraocular pressure decrease (means ± S.E.). The mydriasis is based on 8 animals throughout. Seven of these contribute to the ascending part of the pressure curve and, for technical reasons, only 4 to the descending part.

Majority of the animals did not return to its premydriatic value but remained somewhat wider than the decentralized side by 0.3 to 0.8 mm. (Fig. 3). Bilateral adrenalectomy (n = 3) did not eliminate the difference. Attempts to eliminate it by hexamethonium bromide 10 to 20 mg. per kilogram failed (n = 5). Generally the pupillary effect of hexamethonium in the high doses used was very similar to that of atropine or biperiden. The effect of the drug was particularly inconsistent after the
Table I. The main parameters concerning the postdenervation mydriasis and IOP decrease

<table>
<thead>
<tr>
<th></th>
<th>Onset</th>
<th>Tₜₚ</th>
<th>Width</th>
<th>Duration</th>
<th>Height</th>
<th>Inverse slope (A)</th>
<th>Inverse slope (B)</th>
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<tr>
<td>(A) Mydriasis</td>
<td></td>
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<td></td>
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<tr>
<td>Tₑ (hr.)</td>
<td>14.51</td>
<td>17.05</td>
<td>9.93</td>
<td>18.01</td>
<td>2.78</td>
<td>1.24</td>
<td>2.53</td>
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<tr>
<td>n = 8</td>
<td>±0.21</td>
<td>±0.66</td>
<td>±0.52</td>
<td>±0.89</td>
<td>±0.26</td>
<td>±0.21</td>
<td>±0.43</td>
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<tr>
<td>(B) IOP decrease</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tₑ (hr.)</td>
<td>18.71</td>
<td>20.82</td>
<td>11.74</td>
<td>18.87</td>
<td>6.02</td>
<td>1.50</td>
<td>2.57</td>
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<tr>
<td>n = 7</td>
<td>±0.78</td>
<td>±0.68</td>
<td>±1.63</td>
<td>±1.02</td>
<td>±0.51</td>
<td>±0.27</td>
<td>±0.75</td>
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<tr>
<td>Time interval (B)-(A)</td>
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<td></td>
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<td></td>
<td></td>
<td>4.18</td>
<td>3.81</td>
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<tr>
<td>n = 8</td>
<td>±0.74</td>
<td>±0.82</td>
<td></td>
<td></td>
<td></td>
<td>3.81</td>
<td>3.81</td>
</tr>
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</table>

Significance of the difference: P < 0.001 P < 0.001 not significant not significant comparable significant significant

*To and Tₑ are hours after denervation.

The mydriasis starts 14.5 hours after denervation and reaches the apex plateau 5.5 hours later (8 animals).

The IOP starts to decrease 18.7 hours after denervation and the effect reaches the apex plateau 5 hours later (7 animals all of which contributed also to the mydriasis curve). The ascending limbs of the pressure decrease curve and the mydriasis curve are almost parallel (not significantly different slopes, Table I) with an interval of 4 hours separating them. The pressure decrease curve continues to rise after mydriasis already has reached its maximum and even has started to decrease. The apex plateau of the pressure decrease curve is longer than the mydriatic one. The descending limbs of both curves (Fig. 5, where only animals which contribute to both curves are used) are also reasonably parallel to each other, with an interval of 4 to 6 hours separating them. The slopes of the ascending limbs of the individual curves of the mydriasis and the pressure effect are almost parallel. The slopes of the descending limbs of both curves are also reasonably parallel.

As can be seen from Table I, the width and duration of the mydriasis are 10 and 18 hours, respectively, and those of the pressure effect are 11.74 and 18.87 hours (n = 4). Neither the width nor the duration values of the 2 effects differ signifi-
Fig. 5. The mean curves of the degeneration mydriasis and pressure decrease measured simultaneously on the same 4 animals (means ± S.E.). The line of black triangles represents the data of Sears and Gillis on the appearance of tritiated norepinephrine in the anterior chamber.

Discussion

The degeneration mydriasis. Mydriasis is a constant denervation phenomenon after cervical ganglionectomy in the rabbit. We could not confirm the observation of Hendley and Crombie that it exists only when the animal is startled and that it disappears after adrenalectomy. Neither bilateral adrenalectomy nor hexamethonium bromide 20 mg. per kilogram could prevent or halt the "normal" development of the denervation mydriasis. The mydriasis starts on an average 14.5 hours post operation. That is quite early compared to the other rabbit denervation phenomena, namely, the pressure decrease effect and the vasoconstriction in the ear vessels (unpublished data) which start at 19 hours and 20.5 hours, respectively. Starting time of the mydriasis is rather similar to that of the degeneration contraction of the periorbital muscles of the rat—13 to 15 hours. In the cat nictitating membrane, the start of the contraction is at 20 hours.

Denervation mydriasis has an average duration of 18 hours and not, as was thought before, a very short one (1 to 2 hours). In the rat periorbital muscles the duration is about 12 hours, in the cat nictitating membrane 10 to 14 hours.

Two features of the degeneration contraction curve will now be discussed: (1) that the ascending limb is steeper than the descending limb (this is also so in the periorbital muscles of the rat), and (2) that the curve of the degeneration mydriasis does not fit the norepinephrine depletion curves of the iris, but deviates considerably to the right (Fig. 6).

During the process of degeneration, the nerve terminals lose their ability to take up the released transmitter, resulting in larger amounts of transmitter available to the muscle receptors. This is the basic mechanism of denervation supersensitivity.

No detailed study concerning the denervation supersensitivity during the de-
Fig. 6. The time course of the degeneration mydriasis (integral curve) as compared to the time course of norepinephrine depletion of the iris.

Fig. 7A. The influence of supersensitivity and tone on the mydriasis curve. Schematic representation of our arbitrary assumptions. The bell-shaped curve is the denervation mydriasis. Supersensitivity increases nonlinearily, reaching 32 times at 30 hours. Tone increases as a straight line and reaches 25 per cent of the height of the phenomenon at 30 hours.

Generation mydriasis has been performed. Langham and Langham and Taylor, however, observed a considerable supersensitivity of the denervated rabbit iris to norepinephrine 24 hours after operation.

Figs. 7A, 7B, and 7C demonstrate schematically how the denervation supersensitivity might influence the shape and the magnitude of the mydriasis curve. We have arbitrarily assumed that the supersensitivity increased gradually, as illustrated in Fig. 7A, throughout the phenomenon, reaching 32 times at the end.

One can see that the denervation supersensitivity increases the magnitude of the effect, shifts the whole curve including the
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**Fig. 7B.** Measured mydriasis curve, very thick line; mydriasis sine tone, marked T; mydriasis sine supersensitivity, marked S. Note how the peak of the mydriasis curve moves to the left after division by the supersensitivity factor.

**Fig. 7C.** Comparison of the shapes of the 3 curves. In order to eliminate the influence of size difference, the peaks of all the curves were brought to 100 per cent. The ascending part of the T curve almost coincides with the observed curve. The S curve, corrected for supersensitivity, is steeper over most of its range and it is skewed to the left.

Apex plateau to the right, and changes its shape (Figs. 7B and 7C).

Fig. 7C also shows that the denervation supersensitivity decreases the slope of the ascending limb of the curve, if the slope is expressed in per cent of full effect per unit of time. It also considerably decreases the slope of the descending limb of the curve (except the last 25 per cent of the effect). This could be expected as the denervation supersensitivity develops slowly during the ascending part of the
effect and very rapidly during the descending part\textsuperscript{22, 23} (Fig. 7A). Thus, supersensitivity to the locally released transmitter tends to make the ascending limb steeper than the descending limb.

As was mentioned in the results, in the majority of the animals the pupil did not return to its premydriatic value in the denervated eye after the end of the degeneration contraction, but a difference of 0.3 to 0.8 mm. continued to exist between the denervated and decentralized pupils. Langer and colleagues\textsuperscript{22, 23} have described a tone in the denervated nictitating membrane which was caused by the increased amount of circulating catecholamines in the spinal cat. This tone was found to decrease considerably after bilateral adrenalectomy and was almost eliminated by hexamethonium.\textsuperscript{22, 26} In contrast, the residual tone of the dilator muscle of the denervated iris was influenced neither by bilateral adrenalectomy nor by hexamethonium in high doses.

Figs. 7A, 7B, and 7C demonstrate schematically how a linearly increasing dilator tone would affect the curve of the degeneration mydriasis. (The source of the tone will be discussed presently.) One can see that its influence is quite similar to that of the denervation supersensitivity: It increases the magnitude of the effect and affects its temporal course.

Considering the substantial differences between the residual tone of the denervated iris in the conscious rabbit and Langer's tone in the nictitating membrane of the spinal cat, it seems unlikely that the former is caused only or even mainly by circulating norepinephrine.

Sears and Gillis\textsuperscript{24} have measured the appearance of tritiated norepinephrine in the anterior chamber of the rabbit after denervation (Fig. 5). He has also shown\textsuperscript{27} that as little as 20\,\mu g of intracameral norepinephrine could produce pupillary dilatation in the supersensitive denervated iris.

Considering these observations, he\textsuperscript{10, 24} pointed out the possibility that the transient mydriasis seen after denervation could be caused by the transmitter which accumulates in the aqueous humour.

In fact, considering the resistance of the residual tone of the dilator muscle to hexamethonium and bilateral adrenalectomy, it is very reasonable to believe that the transmitter which accumulates in the aqueous humor and acts on the increasingly supersensitive iris is the main, or even the only, source of transmitter causing the observed residual mydriasis.

**The pressure effect.** Hourly measurements of the pressure decrease effect after cervical ganglionectomy reveal that it starts about 19 hours after denervation and comes to a maximum 5 hours later. The duration of the effect averaged 19 hours. These results corroborate the previous, more infrequent measurements.\textsuperscript{5-7, 10, 16, 18}

The mydriasis curve is a function of the rate of transmitter release. Is this true also for the pressure decrease? Figs. 4 and 5 demonstrate that the pressure continues to decrease after the mydriasis has reached its apex plateau and even after it begins to decline. This speaks in favor of transmitter accumulation in the aqueous as the link between degeneration and pressure decrease. If the pressure decrease had been due to degenerating nerve terminals in the outflow channels, one would have expected a similar time course as for the mydriasis, always assuming that the terminals degenerate approximately equally early in the two structures. There are, however, intervals of 4 hours and 6 hours, respectively, separating the two limbs of the curves.

Fig. 5 summarizes data taken from Sears and Gillis\textsuperscript{24} and our own observations. The transmitter starts to leak out of the nerve endings 14.5 hours after denervation and immediately stimulates adjacent\textsuperscript{15} dilator muscle cells. Spreading through the tissue it starts to leak out of the iris into the aqueous humour. It reaches a measurable concentration after approximately 18 hours. After 19 hours it has approximately 25 per cent of its maximal concentration in
the anterior chamber. In our experiments, the decrease in IOP starts after approximately 19 hours. The agreement in time between the start of the pressure drop and the appearance of considerable amounts of norepinephrine in the anterior chamber is gratifying, but might be fortuitous considering the possibilities of strain differences between the two laboratories. The pressure curve and the norepinephrine curve have different shapes, but the long intervals between the measurements of Sears and Gillis and the experimental errors in both of our data make the significance of the shape difference appear doubtful.

In the following paper we will therefore attempt to estimate (indirectly) the time course of the transmitter concentration and the IOP in the same eye.

REFERENCES

23. Langer, S. Z., Draskoczy, P. R., and Trendel-


