The effect of bretylium on the degeneration mydriasis and intraocular pressure decrease in the conscious rabbit after unilateral cervical ganglionectomy

Giora Treister* and Ernst H. Bárány**

The effect of bretylium on the degeneration mydriasis and the intraocular pressure decrease after superior cervical ganglionectomy was studied in conscious rabbits. The contralateral eye was decentralized. Two injections of bretylium, 10 mg. per kilogram, given prior to the degeneration mydriasis (at the time of the operation and 8 hours later) delayed mydriasis for about 3.0 to 5.5 hours. The intraocular pressure decrease was delayed for 9 hours. The duration of the delayed mydriasis was larger than in the untreated animals. The duration of the pressure effect did not change significantly. The magnitude of the phenomena was not markedly affected by the drug. Possible explanations for the time lag which exists between the degeneration mydriasis curve and the pressure decrease curve are discussed. Bretylium, given during the degeneration mydriasis, caused an early strong dilatation of the denervated pupil, while the decentralized pupil showed a weak response. There was a considerable early pressure decrease in both eyes, significantly larger in the denervated eye. The mydriasis gradually disappeared and reappeared as a second wave about 7 hours after the injection of the drug. The second wave of pressure decrease started about 12 to 15 hours after the injection. The magnitude of the second wave of the mydriasis was similar to that in the untreated group. The second wave of the intraocular pressure decrease was somewhat smaller.

Key words: cervical ganglionectomy, mydriasis, intraocular pressure decrease, bretylium, pharmacodynamics, time factors, sympathectomy, rabbit.

From the Department of Pharmacology, University of Uppsala, Uppsala, Sweden.
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*Work done during leave of absence from Tel-Hashomer Hospital, Tel-Aviv University, Medical School, Israel.
**Reprint requests to Ernst H. Bárány.

In a previous paper, transient mydriasis and intraocular pressure (IOP) decrease after sympathetic denervation were studied. An interval of about 4 hours was found to separate the two phenomena. The parallelism between the phenomena is in agreement with conclusions of several authors, all pointing to the degenerating sympathetic nerve terminals of the iris as the main source of transmitter responsible for the transient IOP decrease, but the expla-
nation of the time interval is not apparent.

Earlier studies have shown that the adrenergic neuron blocker, bretylium, delays the onset of the postdenervation degeneration contraction by postponing the leakage of the transmitter out of the degenerating nerve terminals. This delaying effect was found to differ from the neuron blocking effect of the drug. The effect was always preceded by a sympathomimetic effect, caused by transmitter release from the nerve terminals.

Considering the iris as a common source of transmitter for both the degeneration mydriasis and IOP effect, it seemed worthwhile to study the influence of bretylium on the time course of these phenomena.

Materials and methods

Experimental animals. Eighteen pigmented and 2 albino rabbits of both sexes, weighing 1.7 to 3.5 kilograms, were used. Commercial food pellets and water were provided ad lib. Left preganglionic sympathectomy (decentralization) and right cervical ganglionectomy (denervation) were performed under pentobarbital anesthesia, 30 to 40 mg. per kilogram intraperitoneally. The technique used was that employed by Sears and Bárány.

Technique and measurement. All the observations were made on conscious animals handled with care so as not to irritate them. The technique has previously been described. Briefly, the rabbit was immobilized by a nylon net. The lens was made to fluoresce by ultraviolet light. The horizontal diameter of the pupil was measured in darkness by a spring-bow caliper with fluorescent tips, which were adjusted precisely to the border between the dark edge of the iris and the fluorescent lens. The distance between the tips was then measured with a ruler with interpolation to 0.1 mm. The 2 albino rabbits were measured by the same caliper under ordinary room illumination.

Measurements were repeated at least hourly, except for 4 hours of night sleep. Each pupillary size value was the mean of at least 4 readings in rapid succession. Each single reading varied at the most by ±0.1 mm.

The IOP was measured by a Mackay-Marg electronic tonometer under topical anesthesia, with benoxinate 0.4 per cent (Novesin).

Calculations. The design of the calculation and the graphical representation of the phenomenon were those employed earlier. Each value of the degeneration mydriasis represents the difference \((D)\) between the means of at least 4 readings, in rapid succession, of the denervated and the decentralized pupils. The individual curves were constructed by plotting \(D\)-values against time after denervation. Fig. 1 indicates the measurements that were taken.

![Fig. 1. Graphic representation of the main parameters of the degeneration mydriasis and intraocular pressure decrease.](image-url)
Table I. The main parameters of the degeneration mydriasis and IOP decrease in bretylium-treated Group I and the untreated group*

|                | Group I, bretylium before the start of the degeneration mydriasis. Two successive injections of 10 mg. per kilogram of bretylium, at the time of denervation and 8 hours later, caused a highly significant delay of the onset (T₀) and the T₅₀ₐ point of the degeneration mydriasis by 3.1 and 5.5 hours, respectively (Table I, C). The difference between these delayed values (2.37 ± 1.01) is probably significant (P <0.05) and caused by the smaller slope of the ascending limb of the curve of the treated group (Fig. 2).

The width and duration of the mydriasis effect in the treated animals are longer

The pressure effect was studied similarly. Each value represents the IOP difference between the decentralized and the denervated eye, except where otherwise stated.

Drugs. The drugs used were a one per cent solution of bretylium tosylate in 0.9 per cent sodium chloride (doses refer to salt) and 2.5 mg. per milliliter of biperiden hydrochloride.

Results

The degeneration mydriasis and IOP decrease effect in 8 untreated animals were reported and discussed in a previous paper. The main parameters concerning the effects are summarized in Table I, B and E.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tₐ (hr.)</th>
<th>T₉₅₀ₐ (hr.)</th>
<th>Width (hr.)</th>
<th>Duration (hr.)</th>
<th>Height</th>
<th>Inverse slope₁</th>
<th>Inverse slope₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Mydriasis, treated Group I</td>
<td>17.65 ± 0.33</td>
<td>22.56 ± 0.27</td>
<td>12.03 ± 0.32</td>
<td>23.33 ± 1.40</td>
<td>2.89 mm</td>
<td>1.92 ± 0.28</td>
<td>3.20 ± 0.34</td>
</tr>
<tr>
<td>n = 4</td>
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</tr>
<tr>
<td>B) Mydriasis, untreated group¹</td>
<td>14.51 ± 0.21</td>
<td>17.05 ± 0.66</td>
<td>9.93 ± 0.52</td>
<td>18.01 ± 0.26</td>
<td>2.78 mm</td>
<td>1.24 ± 0.21</td>
<td>2.53 ± 0.43</td>
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<tr>
<td>n = 8</td>
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</tr>
</tbody>
</table>

Bretylium effect:

(A) Difference between A and B
Significance of the difference P<0.001 P<0.001 P<0.005 P<0.005 Not significant Not significant Not significant

(B) IOP decrease, treated Group I
n = 4 n = 4 n = 4 n = 4 n = 4 n = 4 n = 4

(C) IOP decrease, untreated group¹ n = 7 n = 7 n = 4 n = 4 n = 4 n = 7

Bretylium effect:

(F) Difference between D and E
Significance of the difference P<0.001 P<0.001 Not significant Not significant Not significant Not significant Not significant

(C) Difference between bretylium effect on IOP and mydriasis, F and C
Significance of the difference P<0.001 P<0.001 Not significant Not significant Not significant Not significant Not significant

*Bretylium, 10 mg. per kilogram, was injected intramuscularly at time of operation and 8 hours later.

†Inverse slope of the ascending limb (T₉₅₀ₐ - Tₐ).

‡Inverse slope of the descending limb (Tₐ - T₅₀ₐ).
Fig. 2. The mean curves of the delayed degeneration mydriasis and the delayed IOP decrease after bretylium (mean ± S.E.). Filled symbols = mydriasis and open symbols = pressure. The thin lines represent the same phenomena in the untreated group (Fig. 3). Their relative positions are correct, but they have been shifted on the time axis so that the start of the mydriasis curve almost coincides with that of the delayed curve. (Bretylium, 10 mg. per kilogram, was injected intramuscularly at time of operation and 8 hours later.)

than in the untreated animals. This difference is probably significant (Table I, C). A residual mydriasis, a persistent difference between the denervated and the decentralized pupil after the end of the degeneration mydriasis, exists also in the treated animals. It is presumably caused by transmitter which accumulates in the anterior chamber.\(^1\)

As to the pressure decrease effect, Table I, F and Fig. 2 show that bretylium caused a highly significant 9 hour delay of the onset \(T_o\) of the effect. This delay is much longer than in the mydriasis effect. The difference between the delay of the mydriasis and that of the IOP decrease is significant (Table I, G). The slopes of the ascending and descending limbs of the pressure curves were not significantly affected by bretylium (Table I, F).

On the other hand, the effect of bretylium on the duration of the mydriasis is significantly larger than on that of the IOP decrease (Table I, G). In the bretylium treated group, the mydriasis is significantly longer \((P<0.01)\) than the pressure decrease, while this is not so in the untreated animals (Table I, B and E).

**Group II, bretylium during the degeneration mydriasis.** In a group of 4 animals, the injection of bretylium (10 mg. per kilogram) was given 15.5 to 17.5 hours after denervation, that is 2.5 to 3.5 hours after the start of the degeneration mydriasis. The individual curves for a typical experiment are shown in Fig. 3. Ranges of the values observed in the 4 experiments are given in the text. The figure demonstrates two characteristic features of bretylium given during a degeneration contraction—the enhanced sympathomimetic effect and the "splitting" phenomenon. The denervated pupil started to dilate a few minutes after the injection and then dilated much faster than in the ongoing degeneration contraction. The peak difference between the pupils was 3.5 to 5.5 mm. The sympathomimetic effect in the decentralized pupil was very small, 0.5 to 0.6 mm. (Here, and in the following, the 2 figures indicated the range.) The pupil size reached its lowest value, the trough, 4.5 to 5 hours after the injection, close to or somewhat (0.5 mm.) above the starting value. This effect splits the curve into two separate components. The second com-
ponent of the degeneration mydriasis started only 23 to 24 hours after denervation and continued for 7 to 9 hours. The peak difference between the pupils was 2.0 to 3.5 mm. The delay measured from the time of the bretylium injection to the start of the second contraction at the end of the trough was 6.5 to 7.5 hours.

The pressure (Figs. 3 and 5) started to decrease sharply 10 minutes after the injection, simultaneously in both eyes, and came to a minimum 1 to 1.5 hours later (½ hourly measurements). The drop was larger in the denervated eye. The recovery of the pressure was not as complete on the denervated as on the decentralized side, but the shape of the curve was very similar in the two eyes.

A second decrease of IOP, now only in the denervated eye, occurred 30 to 34 hours after denervation and continued for 10 to 12 hours. The pressure effect was delayed by 14 to 16 hours compared to the starting time in the nontreated control group, and by 3 to 5 hours compared to the starting time in animals which had received the bretylium injections prior to the degeneration mydriasis (Group I). The peak pressure difference was 4 to 7 mm. Hg.

**Group III, bretylium before and during the delayed degeneration mydriasis.** In this group of 4 animals, 3 injections of bretylium were given: 10 mg. per kilogram at the time of operation; 10 mg. per kilogram 11 hours later, and 4 mg. per kilogram 19 to 21.5 hours after operation, that is, 2.5 to 3.5 hours after the start of the delayed degeneration mydriasis.

The first 2 injections delayed the onset of the mydriasis by 3 to 4.5 hours until 17.0 to 18.5 hours after denervation.

Because of the ongoing degeneration
Fig. 4. Effect of bretylium on pupil and pressure in Group III. Bretylium, 4 mg. per kilogram, was injected a few hours after the start of a degeneration mydriasis already delayed by previous injections of the drug.

contraction, it is difficult to distinguish a sympathomimetic effect of the third injection on the pupil (Fig. 4), but there may have been one on pressure (Figs. 4 and 5). The peak difference between the pupils (3 to 4.5 mm.) also was larger than in a normal contraction. After the first wave of mydriasis, the pupil contracted, but even at the trough it was always 0.5 to 1 mm. larger than the decentralized one. The second wave of the degeneration contraction (peak difference between the pupils 2 to 3.2 mm.) started from a very short (30 min.) plateau which was absent in 2 cases. The trough occurred 25 to 28 hours post denervation, 4 to 6 hours later than the trough in Group II. This agrees with the time difference (5 hours) between the single injection in Group II (16.5 hours after denervation) and the third injection of this group (19 to 21.5 hours after denervation).

The time interval between the third injection and the start of the second wave of contraction (6 to 8 hours) is very similar to that found after the single injection in Group II. In both cases, the bretylium was given 2.5 to 3 hours after the onset of the degeneration mydriasis whether delayed or “normal.” Rough measurements show that the total area under the mydriasis curve is approximately the same, whether or not it is split by a trough caused by a late injection of bretylium.

After the third injection of bretylium (19 to 21.5 hours after denervation), the pressure started to decrease very fast in both eyes (Figs. 4 and 5). The time course was similar in both eyes, but the effect was significantly larger in the denervated eye. As a whole, the effect in this group was much weaker than in Group II. In both groups (II and III), the pressure in the denervated eye was only partially restored. Then, 32 to 34 hours after denervation, the IOP in the denervated eye started to decrease again reaching a minimum plateau (peak difference 4 to 6 mm. Hg) at
Effect of bretylium in rabbit

36 to 37 hours after denervation. The duration of the pressure decrease was 12 to 14 hours.

Discussion

Group I. The results in Group I demonstrate the delaying effect of bretylium on the onset of the degeneration mydriasis and of the IOP effect. Thus, the delaying effect of bretylium on degeneration phenomena reported previously was fully confirmed.

The ascending limb of the mydriasis curve of the treated group differs from that of the nontreated group (Fig. 2). This may be due to the following: According to Pluchins and associates beta-TM10 (a neuron blocker which is related to bretylium but less efficient in causing the delay effect), delays the leakage of transmitter but does not delay the morphologic degeneration of the nerve terminals or the development of denervation supersensitivity. Hence, it is reasonable to assume that when the delayed degeneration mydriasis starts in the bretylium treated group, denervation supersensitivity skews the curve to the right and decreases the ascending slope.

Another possibility is that the disappearance of the bretylium effect is rate limiting at an early stage of the contraction. This could also explain why the degeneration mydriasis in the bretylium-treated animals lasts longer than in the nontreated animals. The delayed degeneration contraction of the nictitating membrane after beta-TM10 described by Langer was also longer and less steep than the degeneration contraction in nontreated animals, but in the experiments of Lundberg with bretylium in the rat, no such effect was seen.

There is a marked time lag between the mydriasis and the IOP effect. Two mutually not exclusive explanations seem possible: (1) The pressure effect is not simply and only due to the presence of norepinephrine in the aqueous. For instance, part of the pressure effect could be due to reduced formation of aqueous. (2) The difference in time course between pressure and mydriasis is due to a time lag between the development of mydriasis and that of the transmitter concentration in the aqueous. We will now discuss factors affecting this latter time lag.

The mydriasis curve expresses the rate of transmitter release from the nerve endings of the iris dilator and the gradually increasing denervation supersensitivity. Let us first disregard supersensitivity and
Fig. 6. The calculated time-concentration curve of the transmitter in the anterior chamber as compared to the degeneration mydriasis and the IOP decrease curves, in the bretylium group (see text for way of obtaining the time-concentration curve). $M =$ the mydriasis curve, $a =$ the time-concentration curve, taking $k_{out}$ to be 0.0115, $b =$ the time-concentration curve taking $k_{out}$ to be 0.0115 and correcting for supersensitivity. Isolated dots = the IOP decrease curve.

Fig. 7. The calculated time-concentration curve of the transmitter in the anterior chamber as compared to the degeneration mydriasis and the IOP decrease curves in the untreated treated group (Group I). $M =$ the mydriasis curve, $a =$ the time-concentration curve using a $k_{out}$ of 0.0118. Isolated dots = the pressure decrease curve.

also assume that a constant fraction of the released transmitter immediately enters the anterior chamber. Then, the time-concentration curve in the aqueous will be affected by the time course of the release and by the $k_{out}$ of the transmitter. In Figs. 6 and 7, the time-concentration curve in the anterior chamber was obtained by numerical integration: The area under each one hour period under the mydriasis curve
was taken to be the transmitter released during that hour and then allowed to decay with the selected \( k_{\text{out}} \), which was taken to be the same throughout the course of the degeneration.

Fig. 6 refers to the nontreated group and curve \( a \) shows the time course of transmitter concentration in the anterior chamber as it would have been if mydriasis were the correct measure of transmitter release into the aqueous and if aqueous turnover were 50 per cent per hour, \( (k_{\text{out}} = 0.0115 \) which is close to the average normal aqueous flow in rabbits).

Now, in this curve the effect of supersensitivity on mydriasis has been neglected. In a previous paper,¹ we have assumed for illustrative purposes that the supersensitivity factor has a certain time course (Fig. 7)¹ ending with a factor 32 at the end of the normal degeneration mydriasis. If the mydriasis curve is divided by the supersensitivity factor and the integration involving \( k_{\text{out}} = 0.0115 \) is then repeated, curve \( b \) results for the transmitter concentration. It has moved to the left. The isolated points show the time course of the pressure effect; it is clearly delayed with respect to curves \( a \) and \( b \). In order to move the concentration curves to the right, one has to take a very much smaller value for \( k_{\text{out}} \) than the normal. This would cause a more prolonged accumulation of transmitter in the aqueous. A \( k_{\text{out}} \) of about 0.003 brings the peak of curve \( a \) close to that of the pressure effect, and an even lower \( k_{\text{out}} \) of 0.0008 is necessary to accomplish this for curve \( b \). These values are far too low to seem reasonable, especially as Langham and Taylor¹⁶ did not find any measurable change in \( k_{\text{out}} \) of fluorescein 24 hours after denervation, and their ascorbic acid analysis did not indicate any decrease in blood flow through the secretory part of the uvea at that time.

Could it be that the fraction of released transmitter that penetrates to the aqueous is not constant? It is known that in a normal tissue the transmitter is largely taken up again by nerve terminals. From the point of view of the aqueous, it is waylayered, trapped in the terminals of the dilator, and the iris vessels. As the degeneration proceeds this trapping decreases, because terminals lose their uptake ability. A measure of this decrease, as regards the dilator muscle itself, is denervation supersensitivity, for which we have corrected in an arbitrary manner. Now, if from the point of view of the aqueous, trapping decreases according to the same time curve as from the point of view of the dilator fibers, our curve \( a \) is the best one, because the correction factor for the trapping cancels that for the mydriasis. There are, however, reasons to believe that trapping with respect to the aqueous is different and that additional factors are involved. There is monoamine oxidase in the epithelium covering the posterior surface of the iris¹⁰ and this will tend to destroy transmitter on its way to the posterior aqueous. In order to reach the anterior aqueous directly, the transmitter has to diffuse through a vascularized tissue which, according to Eakins and Eakins,³ Langham,¹⁷ and Hendley and Crombie,¹⁸ is hyperemic 24 hours after denervation. Blood flowing in the vessels will remove transmitter. Further, there are vascular terminals which will trap transmitter until they themselves degenerate. Factors of this kind can delay the appearance of transmitter in the aqueous, but whether they are sufficient to explain the time lag between mydriasis and pressure drop is not clear.

In the bretylium experiments of this group, the time lag between mydriasis and intraocular pressure effect was even larger (about 9 hours) than in the control experiments (5 hours.) Aqueous dynamics can be responsible for only about 1 to 2 hours of the time lag (Fig. 7). The hyperemia factor is present at this time too, since bretylium delays the iris hyperemia as well as the mydriasis (unpublished observations). We do not know if and why the factors causing the time lag in the controls are even more effective after bretylium or
if a new component has entered the system in these experiments.

**Group II.** When injected during the degeneration contraction, bretylium caused a stronger sympathomimetic effect in the denervated pupil than in the decentralized one (Fig. 3). The time course and duration of the effect were very similar to those of Lundberg's in the periorbita muscle of the rat. The basic mechanism of the effect is the release of transmitter from the nerve terminals by the bretylium.

Bretylium caused a splitting of the degeneration contraction, only one part was postponed. Summation of the two parts yields roughly a "normal" degeneration contraction effect. The larger sympathomimetic effect in the denervated pupil could be the result either of larger amounts of transmitter released from the degenerating nerve terminals or of the denervation supersensitivity existing already at the time of injection.

The sympathomimetic effect on pressure occurred almost simultaneously with the mydriatic one. At this early stage of the degeneration, the hyperemia is only beginning (unpublished observations). Maybe this explains why pressure followed mydriasis so closely. Part of the pressure drop can also have been due to a blood pressure drop caused by bretylium.

Earlier studies with phenoxybenzamine and reserpine which both release transmitter, have shown that the outflow channels respond with increased facility to the release. Such an increase very probably contributes to the present pressure drop too.

The pressure decreasing effect of bretylium was larger on the denervated side. Whether this is due to supersensitivity of the outflow channels or other targets for the released transmitter or to easier release or to reduced trapping is not clear.

**Group III.** The estimated delay of the mydriasis in this group and in Group II (6 to 7 hours), where the bretylium was injected during the degeneration mydriasis, is much longer than the delay in Group I (3 hours), where the injection was given before the mydriasis. The same is true for the pressure effects. Lundberg's findings in the periorbita muscle of the rat are very similar.

In this group the same delaying effect on the degeneration mydriasis was observed with 4 mg. per kilogram of bretylium given 2.5 to 3.5 hours after the start of the delayed contraction as with 10 mg. per kilogram given 2.5 to 3.5 hours after the start of an undelayed contraction. Both doses are supramaximal, when given well before the undelayed contraction. The end of the degeneration mydriasis occurred 15 to 20 hours after the bretylium in Group III and 13 to 16 hours after bretylium in Group II. Thus, the smaller dose given after previous doses caused a longer delay for the last fibers to degenerate than the single larger dose. This is most easily explained if the injection in Group III simply replenished the bretylium store in some of the fibers.

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