The effects of pilocarpine on the dynamics of aqueous humor in a primate (Macaca irus)

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Albumin labelled with \(^{131}I\) and \(^{125}I\) was used to determine at the same time and in both eyes the rates of conventional and uveoscleral bulk drainage of aqueous humor in cynomolgus monkeys under pentobarbital anesthesia. With \(10^{-4}\) M per liter of pilocarpine in the anterior chamber, the rate of conventional bulk drainage of aqueous humor was \(0.98 \pm 0.19\) \(\mu\)l per minute as compared with \(0.56 \pm 0.12\) \(\mu\)l per minute in the control eye. The rate of uveoscleral bulk drainage was \(0.07 \pm 0.01\) \(\mu\)l per minute in the pilocarpine eye, and \(1.04 \pm 0.14\) \(\mu\)l per minute in the control eye. The mean rate of aqueous humor production in the pilocarpine eye, \(1.05 \pm 0.17\) \(\mu\)l per minute, was significantly lower than that in the control eye, \(1.60 \pm 0.14\) \(\mu\)l per minute. The average increase in facility that was produced by pilocarpine was \(0.109 \pm 0.042\) \(\mu\)l per minute per millimeters of mercury; it was probably significant. The outflow pressure in the pilocarpine eye was calculated to be about \(3.7\) mm. Hg, that in the control eye was about \(2.6\) mm. Hg. The intraocular pressure was \(2.64 \pm 0.71\) mm. Hg higher on the pilocarpine side. Part of the rise in intraocular pressure was due to the reduction in uveoscleral drainage of aqueous humor that caused an increased flow by way of conventional routes. The reduction in aqueous humor formation in the pilocarpine eyes was related to the rise in intraocular pressure, and probably caused by this rise.

It has recently been reported that in both cynomolgus\(^1\)-\(^3\) and vervet monkeys\(^4\) the aqueous humor is drained in part by the conventional routes and in part by previously unknown uveoscleral routes. The purpose of the present communication is to report on the influence of pilocarpine on the rates of drainage by the different routes. The effects on the intraocular pressure and the outflow facility were also determined to elucidate in the same eyes the complex effects of pilocarpine on aqueous humor dynamics.

Methods

Ten cynomolgus monkeys (Macaca irus), five of each sex, weighing 1.9 to 3.7 Kg., mean weight 2.6 Kg., were employed. The animals were anesthetized with intravenous sodium pentobarbital (30 mg. per kilogram of body weight), and 1,500 international units of heparin was given intravenously. A femoral artery was cannulated for blood sampling and blood pressure measurements. The head was placed in a head holder and the animal was kept warm with a heating pad. Both eyes were then cannulated with three cannulas using a needle gun. The cannulas connected the anterior chamber of each eye to a pressure transducer, a reservoir...
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on an electronic balance arrangement for measurements of outflow facility according to Bárány, and a pair of precision syringes that permitted washing through of the anterior chamber without changes in anterior chamber pressure and volume (Fig. 1). A multipoint strip chart recorder (Philips, D 3239) was used for all recordings.

When the intraocular pressure had stabilized after the cannulation, the facility of outflow of both eyes was determined from a measurement of the inflow from the reservoir-balance arrangement of a pressure 7.5 mm. Hg above the resting level. Then the intraocular pressure was re-adjusted to the resting level and the balance arrangement was disconnected by means of a stopcock. The intraocular pressure of each eye was then measured continuously for the next 120 minutes. The mean pressure during this period of time was calculated from 12 readings of the pressure, one taken every 10 minutes. At the start of the 120 minute period, the contents of the left anterior chamber were mixed with the labelled fluid in the syringes by washing 1 ml. of labelled fluid through the anterior chamber to and fro four times. The fluid contained 131I-labelled albumin and pilocarpine. The anterior chamber fluid of the right eye was similarly equilibrated with fluid containing 125I-albumin. The albumin concentration on both sides was about 0.02 percent, and the concentration of pilocarpine was 10^-4 M per liter; that is, about 25 ug per milliliter of pilocarpine-HCl. The basic solution was that described by Bárány, and the handling of the proteins has been described in a previous communication. Immediately after the mixing, 20 ul samples were collected from each syringe system using micrometers fitted to the syringes. In the following, the contents of the anterior chambers were mixed with those of the syringes by washing through to and fro once every 5 minutes at a rate of about 0.5 ml. per minute. Samples were collected from the syringes every 30 minutes and blood samples were collected similarly. After 120 minutes, the last blood sample was collected and the facility of outflow was then determined again on both sides—which took 10 to 15 minutes. The labelled fluid was then washed out of the eyes and the conventional outflow routes by washing 2 ml. of unlabelled fluid through each eye and then connecting the anterior chambers to the reservoirs of the balance systems adjusted to give an intraocular pressure of about 30 mm. Hg. The reservoirs contained unlabelled fluid. After 5 minutes of high-pressure perfusion of the outflow channels, the animals were killed and the contents of the orbits were dissected as in previous experiments.

Assay of radioactive material. After drying, the samples taken were assayed for 131I-albumin and 125I-albumin as described elsewhere. The counting error was less than 3 percent for all samples that appeared to contain more than 0.5 ul of labelled fluid. For blood samples the error was less than 3 percent for an apparent content of 0.1 ul of labelled fluid.

Calculations. In principle, the rates of conven-

Fig. 1. The anterior chamber of each eye was connected to a pressure transducer, to a reservoir that was continuously weighed, and to a pair of precision syringes coupled on a frame in such a way that the total volume within the two syringes was constant when fluid was washed through the anterior chamber from one syringe to the other. For sampling of syringe fluid by means of the micrometer, the tubing from syringe A was clamped and the tubing was temporarily disconnected from the syringe.
tional and uveoscleral flow were determined as in previous experiments. Thus, for the pilocarpine eye, the rate of conventional flow in μl per minute was determined by dividing the amount (in counts per minute) of 131I-albumin recovered in the pertinent albumin space of the animal by the average activity (in counts per minute per microliter) of the aqueous humor and time (120 minutes). The size of the pertinent blood equivalent albumin space was calculated as 7.4 per cent of the weight of the animal. The rate of uveoscleral flow was calculated similarly by dividing the amount of 131I-albumin recovered in the eye and the periocular tissues by the average aqueous humor activity and time (130 to 135 minutes). The average aqueous humor activity in counts per minute per microliter was assumed to equal the mean value for the activity of the 5 samples collected from the syringe system. The flow data for the control eye were calculated similarly from the 131I-albumin data.

The outflow pressure for the aqueous humor in all experiments was provisionally defined as the rate of conventional drainage in microliters per minute divided by the mean for the outflow facility in microliters per minute per millimeter of mercury. This underestimated the true outflow pressure due to the fact that in the facility figure is included a pseudofacility component (see discussion). In preparing the statistics, differences between the pilocarpine eyes and the controls were calculated from paired data, the two eyes of each animal constituting one pair.

Results

During the experiments there was a 10 to 15 per cent fall in radioactivity in the fluid collected from the syringes. The radioactivity of the blood increased almost linearly with respect to both 131I and 125I. The arithmetic mean ± the standard error of the mean for the mean arterial blood pressure at the start was 88.9 ±4.1 mm. Hg, and at the end it was 77.9 ± 3.7 mm. Hg. Miosis on the pilocarpine side was produced during the first washing through with pilocarpine. Table I summarizes the results.

Intraocular pressure. At the start of the experiments the intraocular pressures on both sides were very similar. At the end, the pressure in the pilocarpine eye had increased by an average of 3.6 ± 1.3 mm. Hg, while that on the control side had fallen by an average of 0.7 ± 1.4 mm. Hg. The difference between the two sides, 4.4 ± 1.3 mm. Hg, was statistically significant (p < 0.01).

Facility of outflow. During the experiments the facility changed so that at the end that on the pilocarpine side was 0.109 ± 0.042 μl per minute per millimeter of mercury higher than at the start (before pilocarpine). In the control eye the facility fell by 0.085 ± 0.046 μl per minute per millimeter of mercury. The rise on the pilocarpine side was probably significant (0.05 > p > 0.01). The fall in the control eye was not significant.

Conventional and uveoscleral drainage. The rate of conventional drainage in the pilocarpine eye was, on an average, 0.42 ± 0.08 μl per minute higher than that in the control eye. The difference was significant (p < 0.01). The rate of uveoscleral drainage in the pilocarpine eye was 0.97 ± 0.14 μl per minute lower than that in the control eye. The difference was highly significant (p < 0.001). The rate of aqueous humor production, defined as the sum of the rates of uveoscleral and conventional bulk drainage, was 0.55 ± 0.12 μl per minute lower in the pilocarpine eye.

Table I. Data obtained from 10 animals

<table>
<thead>
<tr>
<th></th>
<th>Pilocarpine eye</th>
<th>Control eye</th>
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<tbody>
<tr>
<td>Starting intraocular pressure (mm. Hg)</td>
<td>8.24 ± 0.84</td>
<td>8.16 ± 0.64</td>
</tr>
<tr>
<td>Starting facility of outflow (μl/min./mm. Hg)</td>
<td>0.348 ± 0.064</td>
<td>0.390 ± 0.070</td>
</tr>
<tr>
<td>Rate of conventional drainage (μl/min.)</td>
<td>0.98 ± 0.19</td>
<td>0.95 ± 0.12</td>
</tr>
<tr>
<td>Rate of uveoscleral drainage (μl/min.)</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Rate of net formation of aqueous humor (μl/min.)</td>
<td>1.05 ± 0.17</td>
<td>1.00 ± 0.14</td>
</tr>
<tr>
<td>Outflow pressure (apparent) (mm. Hg)</td>
<td>3.15 ± 0.72</td>
<td>1.95 ± 0.42</td>
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*M, arithmetic mean; SEM, standard error of the mean.
than in the control eye. The difference was significant (p < 0.01).

Intraocular pressure and rate of aqueous formation. Fig. 2 shows a plot of the difference in rate of aqueous humor production against the mean difference in intraocular pressure between the pilocarpine eye and the control eye. On the assumption of a linear relationship this was described by

$$y = 0.135x + 0.196$$  (1)

where y is the rate of production of aqueous humor on the control side minus that on the pilocarpine side and x is the mean intraocular pressure on the pilocarpine side minus that on the control side. The regression was significant (p < 0.01). Thus, the larger the pressure rise produced by pilocarpine the more was the rate of aqueous formation reduced.

The apparent outflow pressure. The apparent mean outflow pressure was significantly higher (p < 0.01) in the pilocarpine eyes than in the control eyes. The mean difference was 1.21 ± 0.41 mm. Hg.

Discussion

In the present experiments, 10^-4 M per liter of pilocarpine in the anterior chamber produced an increase in intraocular pressure, and tended to increase the facility of outflow. It almost stopped the outflow by way of uveoscleral routes, and it produced an increased outflow by way of the conventional routes. The rate of aqueous humor production was reduced. The reduction was related to the rise in intraocular pressure.

The rates of conventional and uveoscleral flow. It is not clear by which mechanism pilocarpine almost stopped the bulk flow of aqueous humor into the uveoscleral routes, but it seems likely that the contraction of the ciliary muscle that is produced by pilocarpine in comparable concentrations was the main factor. The contraction of the sphincter muscle may also be involved. Still another factor that has to be considered is a change in the filtration rate from the uveal vessels; an increased rate of net filtration probably raises the tissue pressure within the uvea. This may contribute to the stoppage of the aqueous flow by uveoscleral routes.

The average rate of uveoscleral flow in the control eyes, 1.04 ± 0.14 μl per minute, was higher than that reported in a previous series (0.44 ± 0.06 μl per minute), while the average rate of conventional flow, 0.56 ± 0.12 μl per minute, was lower than in the previous experiments (0.80 ± 0.11 μl per minute). The two series are not quite comparable, however. In the present experiments the animals were, on an average, 0.6 Kg. heavier.

Production of aqueous humor. It has been demonstrated elsewhere that in the cynomolgus monkey the rate of aqueous formation is reduced by an artificial increase in intraocular pressure. The mean reduction was about 0.13 μl per minute per millimeter of mercury. In the present experiments pilocarpine produced an in-
crease in intraocular pressure and a reduction in rate of aqueous formation that was related to the pressure rise. It seems very likely, then, that the reduction in rate of formation was caused by the pressure rise. Equation 1 suggests that the reduction was about 0.14 µl per minute per millimeter of mercury; that is of the same order as in the previous experiments. However, other mechanisms may also have contributed to the reduction in the rate of aqueous formation. It is known from Berggren's in vitro studies that in rabbits pilocarpine in low concentrations reduces the rate of shrinking of ciliary processes devoid of blood supply, which indicates that pilocarpine tends to reduce the pumping of aqueous humor from the ciliary processes. It is also known that pilocarpine influences the Diodrast and iodide pump mechanisms in the ciliary processes. Part of the reduced rate in aqueous production in the present experiments may thus have been due to an influence of the drug on the secretion mechanisms.

Becker has reported that pilocarpine increases the rate of aqueous humor turnover in rabbits. If this is due to a real increase in rate of aqueous formation, there seems to be an interesting discrepancy between the two species as regards the effect of pilocarpine.

**The facility of outflow.** The average increase in facility that was produced by pilocarpine in the present experiments was only probably significant, which is in agreement with Bárány's finding in the same species that the average effect of as much as 20 µg of pilocarpine is quite moderate.

As in all facility measurements, it was assumed in the present calculations that the rate of aqueous production was not influenced by the rise in intraocular pressure that was produced to determine the facility. The pressure dependence of the rate of aqueous humor formation mentioned above demonstrates that this assumption is not altogether correct and that there is a pseudofacility component in the facility data. As mentioned, the pressure dependence of the rate of aqueous formation appeared to be about the same in the pilocarpine eyes as in normal eyes; consequently, it seems probable that the facility-increasing effect of pilocarpine was due to a change in facility of the conventional outflow routes and not to a change in the pseudofacility component.

It should be pointed out that, although draining a large part of the aqueous humor, the uveoscleral routes have very little if any facility measured by conventional means. Thus, although the flow through these routes was almost completely stopped by pilocarpine, this had little or no effect on the facility data.

**The intraocular pressure.** In the present experiments, the intraocular pressure was, on an average, 2.64 ± 0.74 mm. Hg higher in the pilocarpine eyes than in the controls. A similar pressure difference has been found both in vervet and cynomolgus monkeys by Bárány (personal communication). Part of the difference in pressure was due to the fact that in the pilocarpine eyes more aqueous humor had to pass out through the conventional routes. Table I shows that, when it was not taken into account that part of the facility in fact was due to suppression of aqueous formation, the mean difference between outflow pressure in the pilocarpine eye and in controls was 1.2 mm. Hg. If it is assumed that pseudofacility on both sides accounted for an average 0.13 µl per minute per millimeter of mercury, it was calculated from the mean outflow data and the average facilities during the experiments that the true figure for the outflow pressure in the pilocarpine eye and in controls was 3.7 mm. Hg and on the control side about 2.6 mm. Hg. The difference in outflow pressure thus remained about the same even when pseudofacility was corrected for. Then, if the pressure in the blood vessels collecting the aqueous humor had been the same on both sides, the intraocular pressure should have been about 1.2 mm. Hg higher on the pilocarpine side than on the other side. The fact that the average difference was more than twice
this value suggests that pilocarpine may have increased the episcleral venous pressure.

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REFERENCES