by an increase in the permeability of the ciliary epithelium as well as possible changes in iris vascular permeability. These changes thereby result in an increase in the measured total outflow facility due to the increase in $C_{\text{p}}$, alone, since $C_{\text{p}}$ is a measure of the permeability of the ciliary epithelium. All the changes caused by PC, therefore, appear to be restricted to the aqueous inflow rather than having any involvement with trabecular, or true, outflow facility.

From the Departments of Ophthalmology and Physiology, Medical College of Georgia, Augusta, Ga. 30902. This study was supported in part by United States Public Health Service Research Grant EY 00863 from the National Eye Institute. Submitted for publication Aug. 5, 1974. Reprint requests: K. Green, Ph.D., Room 3 D 11, R & E Building, Medical College of Georgia, Augusta, Ga. 30902. We thank Mrs. Debbie Graves for her assistance in the preparation of this manuscript. The prostaglandins were generously provided by Dr. J. E. Pike, The Upjohn Company, Kalamazoo, Mich.

Key words: prostaglandins, rabbit, intraocular pressure, total outflow facility, aqueous formation, vasodilation, capillary pressure.

REFERENCES


Adenosine 3',5'-monophosphate increases the outflow of aqueous humor from the rabbit eye. Arthur H. Neufeld, David K. Duiker, Torger Vegge, and Marvin L. Sears.

Direct administration of cyclic-AMP into the anterior chamber increases the outflow facility of the eye for aqueous humor. This is consistent with the hypothesis that catecholamines lower the intraocular pressure of the rabbit eye, at least in part, by a cyclic-AMP-mediated mechanism. This mechanism is active in the outflow channels and increases the rate at which aqueous humor leaves the anterior chamber.

Adrenergic agonists, administered to the eye, decrease intracameral pressure. The mechanism of this response is important because catecholamines may have a role in the physiologic regulation of intracamer pressure and because the clinical use of topical epinephrine is an effective therapy for primary open-angle glaucoma. One of the means by which a decrease in intracamer pressure occurs is an increase in the outflow of aqueous humor. In the rabbit eye, the outflow of aqueous humor responds to catecholamine stimulation; however, the site of action and the mechanism by which adrenergic agonists influence outflow are unknown.

We wish to report what we believe is the next step in a series of events that leads to increased outflow of aqueous humor after an endogenous or exogenous adrenergic stimulus. We have demonstrated that adenosine 3',5'-monophosphate (cyclic-AMP), administered directly into the anterior chamber of the rabbit eye, increases the outflow facility of the eye for aqueous humor.

Methods. Two to three kilogram male albino rabbits were anesthetized with intravenous urethane (25 per cent w/v) and given aspirin (600 mg.) rectally. As a control, three animals were not pretreated with aspirin. The anterior chamber of one eye was cannulated with two needles using the needle gum. One cannula was used for delivery of the drug; the other cannula was connected by saline-filled polyethylene tubing to a transducer and a reservoir and was used to monitor intracamer pressure and to allow perfusion of the eye. Outflow facility was measured by perfusion at two levels of constant pressure, usually $P_1 = 25$ and $P_2 = 30$ mm. Hg. The flow from
the reservoir into the eye at both pressures was
measured by following the decrease in weight of
the reservoir over one to two minutes. All test
compounds were from Sigma (St. Louis, Mo.). To
grossly determine ocular irritation and drug ef-
cficacy, observations of pupil size, blood vessels,
and aqueous flare were made.

Results. Table I lists the values of outflow
facility for the rabbit eye before and after delivery
of the test substance into the anterior chamber.
The delivery of 5 μl of isotonic saline had no
significant effect on outflow facility. Fifty
nanomoles of epinephrine, delivered into the
anterior chamber, caused pupillary dilation and
an increase in outflow facility. Both cyclic-AMP
and N6, 2’-O-dibutyryl cyclic-AMP (dibutyryl
cyclic-AMP) delivered into the anterior chamber
caused large increases in outflow facility of com-
parable magnitude to epinephrine. Although dose-
response relationships have not been fully in-
vestigated, this concentration of cyclic-AMP did
not produce the maximal effect. The increase in
outflow facility in response to cyclic-AMP in ani-
mals not pretreated with aspirin was identical.
Neither cyclic-AMP nor dibutyryl cyclic-AMP had
an effect on pupil size. Adenosine 5’-monophos-
phate (5’-AMP), the inactive metabolite of cyclic-
AMP, caused no change in outflow facility.

Fig. 1 shows the time course of the effect on
outflow facility of 50 nanomoles of 5’-AMP, epi-
nephrine, or cyclic-AMP delivered into the anterior
chamber at time 0. 5’-AMP produced no sig-
nificant change in outflow facility for up to four
hours. When epinephrine or cyclic-AMP was
administered, outflow facility increased and re-
mained elevated for at least four hours. The
magnitude of the responses to epinephrine and
cyclic-AMP was approximately equal. The pupil-
ary dilation to the administration of epinephrine
lasted approximately one hour.

Discussion. Many studies of the regulation of
intraocular pressure have utilized the rabbit eye.
However, it is easily irritated, and consistent
results are difficult to obtain. Cannulation and
manipulation of the eyes in these experiments was
relatively atraumatic, but the presence of two
needles in the anterior chamber for several hours,
and the perfusion of saline and pharmacologically
active agents from these needles, may become
irritating to the eye. The irritative response of the
eye to trauma is often mediated by prostaglandins
and we have previously demonstrated that aspirin
can protect the eye from acute, post-traumatic
irritation. To minimize the hyperemia, miosis,
and aqueous flare that occurs with prolonged ex-
perimentation, our animals were pretreated with
aspirin, an inhibitor of the synthesis of prostaglan-
dins. We believe this will be of value in research
involving manipulation of the rabbit eye and
perhaps other organs as well. Pretreatment with
aspirin does not alter outflow facility nor the
response to exogenous cyclic-AMP. No difference
in control values of outflow facility of rabbits,
pretreated or not treated with aspirin were ob-
served, and our values for outflow facility in the
presence of aspirin were similar to those values
previously reported in the literature in the
absence of aspirin. In addition, a doubling in the
value of outflow facility occurred in response to

Table I

<table>
<thead>
<tr>
<th></th>
<th>C0</th>
<th>C1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (6)</td>
<td>0.20 ± 0.02</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Epinephrine (9)</td>
<td>0.28 ± 0.01</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>Cyclic-AMP (11)</td>
<td>0.22 ± 0.02</td>
<td>0.46 ± 0.04</td>
</tr>
</tbody>
</table>
| Dibutyryl cyclic-
  AMP (11)       | 0.25 ± 0.03 | 0.50 ± 0.06 |
| 5’-AMP (12)     | 0.25 ± 0.02 | 0.24 ± 0.02 |

C0 is the outflow facility with two cannulas in the anterior
chamber, approximately 30 minutes before administration
of the test substance. C1 is outflow facility approximately
30 minutes after delivery of 5 μl of saline, either as a
control or containing 50 nanomoles of the drug. All values are
the mean ± S.E.M. The number of animals tested is in
parentheses.
cyclic-AMP in rabbits not pretreated with aspirin; identical to the response in pretreated animals. Cyclic-AMP is in the aqueous humor of untreated rabbit eyes at a concentration of approximately 25 nmoles per liter. The pharmacologic response to topical epinephrine of decreased intraocular pressure is associated in vivo with a three- to fourfold increase in cyclic-AMP in the aqueous humor of the anterior chamber. Administration of cyclic-AMP into the anterior chamber causes a decrease in intraocular pressure. Cyclic-AMP is found in various tissues which line the anterior chamber, such as the cornea, iris, and sclera-trabecular tissue. These tissues, when incubated in vitro, increase their cyclic-AMP level in response to adrenergic agonists. In addition, when increased or decreased responsiveness of the eye to epinephrine is produced experimentally, parallel changes in the responses of intraocular pressure and cyclic-AMP in the aqueous humor are found.

Our early work on cyclic-AMP accumulation in the aqueous humor was confirmed by Radius and Langham studying the effects of norepinephrine. Although they agreed that cyclic-AMP mediates the decrease in intraocular pressure in response to adrenergic agonists, they did not feel that their results supported one of our alternative mechanisms; that cyclic-AMP in the aqueous humor was the actual mediator.

Considering the results reported in this paper and the observations cited above, we conclude that cyclic-AMP, at least in part, mediates the decrease in intraocular pressure in response to adrenergic agonists by influencing the outflow of aqueous humor. Previously, similar preliminary results on outflow were reported using dibutyryl cyclic-AMP. The involvement of cyclic-AMP in this phenomenon, and the duration of its effect, underlines the need to consider a cellular response as part of the mechanism by which catecholamines influence the outflow of aqueous humor. Cyclic-AMP may regulate changes in fluid movement, either intracellularly or extracellularly through phosphorylation of membranes, subcellular reorganization, or alterations of metabolic processes. Although other responses may also play a role, cyclic-AMP-mediated effects may explain both the early and the prolonged actions of catecholamines on outflow.

The site of action of catecholamines and the "second messenger," cyclic-AMP, to increase outflow may include the endothelium of the trabecular meshwork, the juxtanaculcular tissue, the lining of the canal of Schlemm, and/or the blood vessels. However, the transient changes in vessel diameter in response to adrenergic agonists cannot be of great importance since the effects of a single administration of epinephrine or cyclic-AMP on outflow are relatively long lasting. Further work is in progress on the site, duration, and mechanism of action by which cyclic-AMP causes an increase in the outflow of aqueous humor.

We gratefully acknowledge the skillful technical assistance of Deborah J. Whitman.

From the Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Conn. This work was supported in part by United States Public Health Service Grant Nos. EY-00237, EY-00785, EY-00943, and Contract No. N01-EY-1-2512. Submitted for publication June 17, 1974. Reprint requests: Dr. A. H. Neufeld, Yale University School of Medicine, 333 Cedar St., New Haven, Conn. 06510.

Key words: cyclic-AMP, outflow facility, aqueous humor, intraocular pressure, rabbit, catecholamines.

REFERENCES