Study of Central Regulation of Intraocular Pressure
Using Ventriculocisternal Perfusion

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The ability of hypoosmotic solution, prostaglandin E₁ (PGE₁) and clonidine to influence intraocular pressure (IOP) by a central mechanism was studied using the technique of ventriculocisternal perfusion in conscious rabbits. IOP remained unchanged during the perfusion of 150 mOsm artificial cerebrospinal fluid. IOP rapidly increased by 15 mmHg during the perfusion of PGE₁ at the dose of 1 or 3 µg/min. However, when PGE₁ was perfused intravenously at the dose of 1 µg/min, a similar IOP response was observed. Furthermore, during the ventriculocisternal perfusion of PGE₁ a significant systemic absorption occurred. These observations indicate that the ocular hypertension during the ventriculocisternal perfusion of PGE₁ is primarily due to the peripheral action of systemically absorbed PGE₁. IOP gradually decreased by 3 mmHg during the ventriculocisternal perfusion of clonidine at the dose of 0.1 or 0.33 µg/min. Intravenous perfusion of clonidine at the same doses did not change the IOP. These results indicate that clonidine can lower IOP by a centrally mediated mechanism. Ventriculocisternal perfusion of clonidine (0.1 µg/min) in rabbits with unilateral superior cervical ganglionectomy lowered IOP in both eyes, indicating that ocular adrenergic innervation does not participate in this centrally mediated IOP response. However, the cardiovascular parameters of anesthetized rabbits were altered by the ventriculocisternal perfusion of clonidine (0.1 µg/min), suggesting that a change in systemic hemodynamics is involved in the central IOP effect of clonidine. Invest Ophthalmol Vis Sci 26:136–143, 1985

The site of action of most antiglaucoma agents which decrease elevated intraocular pressure (IOP) is believed to be limited to the eyeball itself. A possible pharmacologic mechanism in the central nervous system (CNS), in addition to the local mechanism, to decrease IOP by certain agents, such as clonidine,¹² timolol¹³ and cannabinoids,⁴,⁵ has been postulated, but there is a lack of direct evidence.

Over the past decade, microinjection through a cannula into the third cerebral ventricle of a conscious rabbit⁶ has served as the model for studying the central, pharmacologic effect on IOP. A variety of substances (hypoosmotic and hyperosmotic agents, prostaglandin E₁, arachidonic acid, calcium, and substance P) injected into the third ventricle can cause IOP alterations.⁶-⁹ This implies that a central locus influencing IOP may exist in the area of the third ventricle.

In our laboratory, the use of third ventricular microinjection has encountered some difficulties. We have observed that the injection itself causes a transient, inconsistent IOP alteration. In addition, there is no simple technique for defining the location of the cannula in the brain; therefore, the exact position of the cannula cannot be routinely verified in the postoperative rabbit.

We report here an alternate model for investigating the IOP response to centrally administered agents by using the technique of ventriculocisternal perfusion. This technique allows the cerebral ventricular system, except one lateral ventricle, to be perfused and has been developed successfully in conscious goats,¹⁰ dogs,¹¹ cats,¹² and rabbits.¹³

We used this model to study the ability of three possible centrally acting agents, a hypoosmotic solution,⁶ prostaglandin E₁ (PGE₁),⁷ and clonidine,¹² to induce ocular hypertension or hypotension. Then, we determined if the IOP alteration during the ventriculocisternal perfusion was due to a peripheral action of circulating test agents, systemically absorbed during the perfusion. Finally, the involvement of ocular
adrenergic innervation and systemic hemodynamics in the centrally mediated IOP response were studied.

**Materials and Methods**

Adult New Zealand albino and pigmented rabbits (3-5 kg) were used. Rabbits were cared for and treated in accordance with the ARVO Resolution on the Use of Animals in Research.

**Cannulation**

The surgical procedure of inserting cannulae consisting of two lateral ventricular and one cisternal guide tubes for the ventriculocisternal perfusion followed the description by Moir and Dow with modifications.

Rabbits were anesthetized with intravenous pentobarbital (30 mg/kg). At the beginning of the operation, an antibiotic, 250 mg cephapirin, was injected intramuscularly. The subcutaneous tissue of the scalp and back of the neck were infiltrated with 1% lidocaine containing 0.001% epinephrine. Through a midline incision the temporalis muscle and periosteum overlying the vault of the skull were reflected laterally. A stereotaxic instrument (model 900, David Kopf Instruments; Tujunga, CA) was used to locate the points for placement of the guide tubes. Two 3-mm holes, 6 mm posterior to the coronal suture and 5.5 mm lateral to the sagittal suture, were made using a dental drill (EMESCO, Englewood, NJ; with no. 801 and 815 diamond points). Care was taken to avoid damaging the dura. The ventricular guide tubes (David Kopf Instruments, model 201 cannula without shaft) were screwed firmly into place.

The incision was extended caudally and the nuchal ligament was incised longitudinally. Muscles were retracted laterally to allow the posterior part of the occipital bone and the atlantooccipital membrane to be visualized. The periosteum overlying the superior aspect of the occipital protuberance was removed and a 1.6-mm hole was drilled through the occipital bone, pointed toward the atlantooccipital membrane. A cisternal guide tube (blunt 18G needle) was pushed firmly into place and fixed with instant cyanoacrylate glue on the occipital bone. The curved tip of the guide tube allowed the cisternal tube to approach the cisterna magna. When the glue had hardened, the nuchal muscle was approximated and the skin closed.

**Ventriculocisternal Perfusion**

Perfusion was not performed until at least 1 week after the surgery and at least 1 week of rest was allowed between perfusions on individual rabbits.

The arrangement of the apparatus for ventriculocisternal perfusion is shown in Figure 1. Conscious, postoperative rabbits were conditioned in a box restrainer. A ventricular tube (beveled 22G stainless steel thin-wall tube, Small Parts Inc.; Miami, FL) was slowly inserted into one ventricular guide tube. The correct placement in the lateral ventricle was observed by a rapid fall of perfusate along the polyvinyl tubing connected to the tube. A cisternal tube (23G stainless steel thin-walled tube) was inserted into the guide tube and was advanced slowly into the cisterna magna until free cerebrospinal fluid (CSF) flow occurred, which indicated the correct placement. These tubes were connected to a Gilson minipuls-2 infusion apparatus by flexible polyvinyl tubing (i.d. 0.51 mm). Sterile perfusate at 37°C was infused through the ventricular tube and withdrawn through the cisternal tube. In preliminary studies, perfusion with artificial CSF at various rates between 30 μl/min and 60 μl/min caused no change in IOP. Throughout the entire study, a constant rate of 30 μl/min was used. At this perfusion rate, approximately 10 min were needed for a perfusate traveling through the cerebral ventricles.

Five cannulated rabbits were perfused with artificial CSF for 5 hr to serve as the control group for relevant experiments. IOP was monitored with a modified Digilab pneumotonometer calibrated for rabbit eyes. For each measurement, three successive IOP readings were taken, and the average was recorded. Topical 0.5% proparacaine HCl was used as local anesthetic.
Three test agents, hypoosmotic artificial CSF, PGE₁, and clonidine, were studied. The standard protocol for each experiment included baseline IOP measurements, one hr perfusion with artificial CSF, a period of perfusion with the test agent in artificial CSF and, finally, a washout period of 1 or 1.5 hr with artificial CSF. Diluted artificial CSF (150 mOsm) and PGE₁ (0.3, 1, and 3 μg/min) were perfused for 1 hr. Clonidine (0.01, 0.033, 0.1, and 0.33 μg/min) was perfused for 2 hr. Five to seven rabbits were used at each dose. Bilateral IOPs were measured every 15 min throughout the perfusion. The average bilateral IOPs in each experimental group and the control group were compared using Student's t-test. The level of significance for difference was set to be P < 0.05 and N = rabbits.

Systemic Absorption

There is constant turnover of CSF, which has been estimated at approximately 1.5% (6 μl/400 μl) of CSF per min in rabbits. Additional systemic absorption of test agents can occur if other pharmacokinetic factors, such as active transport or breakdown of the blood–brain barrier, participate. Thus, during ventriculocisternal perfusion, part of the test agent would appear in the systemic circulation and perhaps a peripheral IOP response to circulating test agents, such as PGE₁, would occur.

The absorption of PGE₁ into the circulation during the ventriculocisternal perfusion was determined by comparing the amount of labeled PGE₁ in the plasma following equivalent ventriculocisternal and intravenous perfusions. Using the standard protocol, [3H]-PGE₁ (approximately 10 μCi/ml in the perfusate) was perfused centrally at the dose of 1 μg/min in four rabbits. When [3H]-PGE₁ had been perfused centrally for 30 min, the radioactivity in the plasma was determined using a Beckman LS-8100 scintillation counter. This value was compared with the amount of radioactivity following the equivalent intravenous perfusion of [3H]-PGE₁ at the dose of 0.5, 1, and 2 μg/min for 30 min, obtained in groups of three rabbits.

Intravenous Perfusion

PGE₁, at the dose of 0.3 and 1 μg/min, and clonidine, at the dose of 0.1 and 0.33 μg/min, were perfused intravenously via the marginal ear vein according to the same protocol as for ventriculocisternal perfusion. Five conscious rabbits, some had implanted cannulae, were used for each dosage. IOPs in both eyes were monitored and compared with those obtained during ventriculocisternal perfusion.

Ocular Adrenergic Denervation

Five rabbits underwent unilateral superior cervical ganglionectomy under general anesthesia. The whole ganglion and part of the postganglionic fiber were removed. Two weeks after surgery, the adrenergic denervation was confirmed by the development of miosis and by testing the pupillary response to 50 μl topical 1% hydroxyamphetamine. At least 1 more week elapsed before cannulae for central perfusion were implanted. Ventriculocisternal perfusions of 0.1 μg/min clonidine were performed in these rabbits followed the standard protocol described above.

Blood Pressure and Heart Rate

We monitored the cardiovascular parameters during the ventriculocisternal perfusion of clonidine (0.1 μg/min) in six anesthetized rabbits (25% urethane, 1.5 g/kg). Prior to the ventriculocisternal perfusion, the femoral artery was cannulated and then blood pressure and heart rate were measured using a Grass polygraph (model 7D). Bilateral IOPs were measured as above except that no local anesthetic agent was given. The protocol of ventriculocisternal perfusion of clonidine in these anesthetized rabbits followed that used in conscious rabbits.

Materials

Clonidine, prostaglandin E₁ and urethane were from Sigma Company; and [5,6-3H(N)]-prostaglandin E₁ was from New England Nuclear. Hydroxyamphetamine hydrobromide was from Smith Kline and French Laboratory.

Results

Approximately 80% of the cannulated rabbits were suitable for ventriculocisternal perfusion, and up to eight perfusions were conducted in individual rabbits. Ventriculocisternal perfusion with artificial CSF alone did not cause significant IOP alterations. However, a small, transient IOP increase (2 mmHg on average) was observed 15 min after beginning the perfusion. No significant IOP change occurred during the perfusion of 150 mOsm artificial CSF (Fig. 2).

Prostaglandin E₁

A significant bilateral IOP increase (15 mmHg on average) was observed in all eyes with the central perfusion of PGE₁ at the doses of 1 or 3 μg/min. With perfusion of PGE₁, at the dose of 0.3 μg/min, a significant IOP increase greater than 10 mmHg was
observed in three of 10 eyes. One other eye had a moderate IOP increase (5 mmHg), and six other eyes had little or no IOP response. Central perfusion of PGE$_1$ at doses lower than 0.3 $\mu$g/min did not cause any IOP change.

The average IOP change for central perfusion of 0.3 and 1 $\mu$g/min PGE$_1$ is shown in Figure 3. In those eyes with significant IOP alterations, IOP increased within 15 min, and the peak of ocular hypertension occurred at about 45 min after the administration of PGE$_1$ began. No significant IOP changes occurred if PGE$_1$ was perfused centrally for only 5 or 10 min. The alteration of IOP was reversible; IOPs gradually returned to normal values when the perfusate no longer contained PGE$_1$.

When $^3$H-PGE$_1$ had been perfused centrally at the dose of 1 $\mu$g/min for 30 min, the average radioactivity in 1 ml plasma was 3561 cpm. The average radioactivity in 1 ml plasma following the intravenous perfusion of $^3$H-PGE$_1$ at the dose of 0.5, 1, and 2 $\mu$g/min for 30 min was 2332, 3637, and 7220 cpm, respectively. Thus, the same amount of radioactivity from central perfusion of $^3$H-PGE$_1$ at the dose of 1 $\mu$g/min was matched by an intravenous perfusion at the dose of approximately 0.9 $\mu$g/min. Apparently there was significant systemic absorption of centrally administered PGE$_1$, in addition to the amount acquired by the normal turnover of CSF.

When PGE$_1$ was perfused intravenously at the dose of 1 $\mu$g/min, a rapid IOP increase (15 mmHg on average) was observed in all eyes (Fig. 4). Intravenous perfusion of PGE$_1$ at the dose of 0.3 $\mu$g/min caused a significant increase of IOP in four of 10 eyes. The average IOP changes during intravenous and ventriculocisternal perfusions at these two doses of PGE$_1$ were similar. The rise and the return of IOP during the intravenous perfusion of PGE$_1$ occurred over a shorter period of time than those during the ventriculocisternal perfusion.
Fig. 5. The IOP response during the ventriculocisternal perfusion of clonidine. Each point is the mean IOP in both eyes ±SEM for five rabbits (Ο control experiment without clonidine; ▽ 0.033 μg/min clonidine; △ 0.1 μg/min clonidine).

Clonidine

A significant bilateral decrease of IOP of approximately 3 mmHg occurred during ventriculocisternal perfusion of clonidine at the dose of 0.1 and 0.33 μg/min. The IOP response to the central perfusion of clonidine at these two doses were similar. Lower doses of clonidine (0.01 and 0.033 μg/min) did not cause any significant response. Figure 5 shows the IOP responses during the ventriculocisternal perfusion of clonidine at the dose of 0.033 and 0.1 μg/min. The ocular hypotension induced by centrally administered clonidine developed slowly, reaching a maximum in 90 min. The decrease in IOPs reversed when the perfusate no longer contained clonidine.

Intravenous perfusion of clonidine at the dose of 0.1 or 0.33 μg/min caused no IOP change. Figure 6 shows the IOP responses during the intravenous and equivalent ventriculocisternal perfusion of clonidine at the dose of 0.1 μg/min.

Fig. 6. The IOP response during the ventriculocisternal and equivalent intravenous perfusion of 0.1 μg/min clonidine. Each point is the mean IOP in both eyes ±SEM for five rabbits (Ο ventriculocisternal perfusion of CSF only; △ ventriculocisternal perfusion of clonidine; □ intravenous perfusion of clonidine).

Fig. 7. The IOP response during the ventriculocisternal perfusion of 0.1 μg/min clonidine in rabbits with unilateral ocular adrenergic denervation. Each point is the mean ± SEM for seven rabbits (△ IOP response in the innervated eye during the perfusion of clonidine; □ IOP response in the denervated eye during the perfusion of clonidine; O ventriculocisternal perfusion of CSF in intact rabbits [N = 5]).

Ventriculocisternal perfusion of clonidine at the dose of 0.1 μg/min was performed in five rabbits with unilateral ocular adrenergic denervation. Clonidine caused a significant IOP decrease in both the innervated eye and the denervated eye. The magnitude and the time course of IOP alterations in both eyes were similar (Fig. 7).

In urethane anesthetized rabbits, ventriculocisternal perfusion of clonidine at the dose of 0.1 μg/min caused a significant ocular hypotension (Table 1). A longer time was needed for IOP to recover in anesthetized rabbits compared with conscious rabbits. Compared with the perfusion of artificial CSF only, ventriculocisternal perfusion of clonidine (0.1 μg/min) caused significant decreases of systolic blood pressure, diastolic blood pressure and heart rate (Figs. 8, 9). These alterations by clonidine were reversed when the perfusate no longer contained clonidine.

Discussion

Ventriculocisternal Perfusion

In this study, we have demonstrated that both ocular hypertension and ocular hypotension can be induced by ventriculocisternal perfusion with specific test agents. A centrally mediated IOP response can be differentiated from a peripheral IOP response due to a systemically absorbed test agent by comparing the dose-response curves following ventriculocisternal and equivalent intravenous perfusions and by determining systemic absorption of the appropriate tracer. Our data indicate that ventriculocisternal perfusion...
Table 1. Change of IOP during ventriculocisternal perfusion of clonidine 0.1 µg/min in urethane anesthetized rabbits*

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<th>Control</th>
<th>Clonidine</th>
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<tr>
<td></td>
<td>Initial IOP (mmHg)</td>
<td>0.5 hr</td>
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<tr>
<td></td>
<td></td>
<td>(CSF)</td>
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<tr>
<td>Control</td>
<td>19.3</td>
<td>0.7</td>
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<tr>
<td></td>
<td>(0.8)†</td>
<td>(0.9)</td>
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<tr>
<td>Clonidine</td>
<td>19.6</td>
<td>0.1</td>
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<td></td>
<td>(1.6)</td>
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* Data were calculated using bilateral IOPs from six rabbits.
† P < 0.05 Student’s t-test, compared with the control.
‡ Values in the parentheses are the SEM.

is a useful technique for studying central, pharmacologic IOP effects.

The ventriculocisternal perfusion technique differs from the technique of third ventricular microinjection in several aspects. Using ventriculocisternal perfusion, physical damage to the brain tissue, especially the area surrounding the third ventricle, is apparently minimal. In addition, the physiologic conditions in the ventricles can be altered easily by changing the perfusate. Therefore, as shown in this study, data from each perfusion can have an internal control, and an experiment can be performed in a strictly controllable fashion. However, using the third ventricular microinjection technique, one selects a specific location for microinjection, and a high local concentration of test agent can be obtained, which is difficult to achieve with the ventriculocisternal perfusion. Each of these factors may contribute to some of the discrepancies in results from ventriculocisternal perfusion and third ventricular microinjection.

The technique of ventriculocisternal perfusion should be used carefully. Because the rabbit must be restrained during the period of perfusion, its back and legs should be carefully protected. Occasionally, rabbits must be sacrificed due to spinal injuries (approximately 5%), especially those injuries associated with the motor reflex to the misplacement of the cisternal tube. Thus, a series of experiments on the same rabbit may be difficult to complete. Furthermore, the outflow in the cisternal tube may become blocked by a clot. Increasing the rate of perfusion may help decrease the incidence of blockage.

Central Regulation of IOP

There is clinical and experimental evidence demonstrating that hypothalamic lesions are frequently
accompanied by changes in IOP. Electrical stimulation of various portions of the diencephalon causes IOP alterations in rabbits and cats. Furthermore, stimulation of the cervical sympathetic trunk in rabbits and cats causes significant IOP changes. These observations imply possible central nervous influences on IOP.

A significant ocular hypertension, a rise of IOP of more than 6 mmHg for 10 min, occurs following injection of 100 µl distilled water into the rabbit third ventricle. This alteration occurs without changes in systemic fluid dynamics, such as blood pressure and osmolarity, suggesting that the IOP response is mediated via the nervous system. The efferent pathway of the IOP response to the third ventricular microinjection of distilled water and to the oral administration of water is apparently associated with the optic nerve because transection of the optic nerve eliminates the response. However, there was difficulty in repeating the observation that cutting the optic nerve decreases the IOP response to orally administered distilled water.

In our laboratory, to confirm the induction of ocular hypertension with microinjection of 100 µl distilled water via the cannula in the third ventricle, was not successful. Only a small, inconsistent, IOP alteration (less than 1 mmHg) was observed. Both this observation and our negative result from the ventriculocisternal perfusion of 150 mOsm artificial CSF do not support the concept of a specific ocular hypertensive response to intracranial hypoosmotic conditions.

Ocular hypertension induced by PGE₁ has been demonstrated by both the third ventricular microinjection and the ventriculocisternal perfusion. A maximal rise of IOP of 4 mmHg followed microinjection of 10 µg PGE₁ into the rabbit third ventricle. An IOP increase of 15 mmHg occurred during ventriculocisternal perfusion of PGE₁ at the dose of 1 µg/min. We demonstrated that there was a significant systemic absorption of centrally perfused PGE₁ and that the IOP response following central and intravenous perfusions of PGE₁ at this dose were essentially identical. We conclude that the ocular hypertension following central perfusion of PGE₁ results primarily from the peripheral effects of circulating PGE₁. A central mechanism by which PGE₁ increases IOP during the ventriculocisternal perfusion would play only a minor role, if any. However, it seems possible that a high concentration of PGE₁ in the third ventricle, achieved by microinjection, can induce ocular hypertension via an undefined central mechanism.

Clonidine, an α₁- and α₂-adrenergic agonist, is used clinically for treating systemic hypertension and presumably works via a centrally mediated mechanism. Topical clonidine decreases IOP in the treated eye and the contralateral, untreated eye in both healthy persons and glaucoma patients. The response in the contralateral eye is approximately 50% of that in the treated eye. Part of this ocular hypotensive effect results from local vasoconstriction by the α₁-adrenergic action of clonidine. Stimulation of ocular α₂-adrenergic receptor which limits the release of neurotransmitter may also contribute to the local IOP effect of clonidine. Several laboratory experiments suggest an additional, centrally mediated mechanism. For example, in anesthetized cats a significant shift of the dose-response curve occurred when comparing clonidine injection via the vertebral artery to intravenous injection.

In our study, the IOP responses following ventriculocisternal and intravenous perfusions of clonidine were distinguished clearly, demonstrating a centrally mediated IOP response. This centrally mediated IOP response was unaffected by ocular adrenergic denervation, indicating that the efferent ocular adrenergic nerves are not involved in the IOP response of centrally acting clonidine. Another study showed that the ocular hypotensive action of topical clonidine in conscious rabbits was abolished by ocular adrenergic denervation, which was probably related to the peripheral action of topical clonidine.

In normal human eyes, topical 0.125% clonidine can cause a significant contralateral ocular hypotensive effect and a significant decrease of systemic blood pressure. In this study, we show that the cardiovascular parameters are affected by ventriculocisternal perfusion of clonidine at the dose effectively causing a central IOP response. This strongly suggests that alterations of cardiovascular parameters may mediate the central IOP effect of clonidine. However, since the relationship between the change of IOP and the alterations of cardiovascular parameters has not been established quantitatively, we cannot rule out the possibility that another mechanism for clonidine exists, working at the same time course as those central mechanisms on the cardiovascular parameters, to cause a centrally mediated IOP response.

Key words: clonidine, hypoosmotic solution, intraocular pressure, prostaglandin E₁, ventriculocisternal perfusion

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