The intraocular pressure response of conscious rabbits to clonidine

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A study has been made of the time courses of the pupillary and intraocular pressure responses of conscious rabbits to clonidine administered either topically or intravenously. Topical unilateral application of clonidine caused transient pupil dilatation and a biphasic intraocular pressure response; an initial hypertensive response preceded a hypotensive phase lasting several hours. Pupillary and hypertensive responses were absent in the untreated eye, but there was a rapid decrease of intraocular pressure. Intravenous administration of clonidine caused an immediate and large decrease of intraocular pressure in both eyes. Phenoxymethylamine given intravenously inhibited the pupillary dilatation and the hypertensive responses to clonidine. The role of efferent adrenergic neuronal activity in mediating the local biphasic pressure response was studied in rabbits with unilateral precervical and postcervical sympathectomy. The results showed the hypotensive response to be dependent on an intact adrenergic innervation of the ocular tissues.

Key words: clonidine, biphasic pressure response, phenoxybenzamine, adrenergic denervation

Clonidine (2-(2,6-dichlorophenylamino) 2-imidazoline hydrochloride; ST-155; Catapres, Glaucomes) is a chemical analog of the adrenergic neuronal blockers, phentolamine and tolazoline; it has been used to treat essential hypertension,1,2 migraine,3 and glaucoma.4-9

Despite more than 9 years of clinical experience with the drug in the treatment of arterial hypertension, its mechanism of action remains unclear.7-9 The hypotensive effect of clonidine is believed to result from the rapid passage of the drug through the blood-brain barrier and to the stimulation of central α-adrenergic receptors resulting in decreased efferent sympathetic and increased vagal activities.10-14 A transient direct sympathomimetic action of clonidine is seen in its peripheral vasoconstrictor action, which is blocked by phenoxybenzamine.7-9

Attempts to characterize the ocular response to clonidine in experimental animals have been few. Hoenke15-16 reported that clonidine solutions applied topically to anesthetized rabbits decreased the intraocular pressure of the treated but not the untreated eyes. Sayegh and Weigel17...
found that an intravenous injection of clonidine in anesthetized rabbits caused the characteristic biphasic change in blood pressure, a significant decrease of intraocular pressure, and a marked decrease of blood flow in the common carotid artery. Recently, Bill and Heilmann reported on the immediate ocular response of anesthetized monkeys and cats to intravenous clonidine. In monkeys, clonidine caused a small decrease in outflow facility, had little effect on intraocular pressure, and was without effect on the rate of aqueous humor formation. In cats, intravenous clonidine reduced ocular blood flow and decreased intrascleral venous pressure, which the authors interpreted to be due to local vasoconstriction.

In this study, we deal with the time course of the pressure response of conscious rabbits to clonidine applied topically and injected intravenously. A biphasic pressure response was found in the treated eyes and a monophasic pressure response in the contralateral eyes. The role of central and peripheral adrenergic mechanisms in mediating the biphasic pressure response has been elucidated with surgically induced adrenergic denervation and the α-adrenergic receptor blocker, phenoxybenzamine.

**Methods**

Experiments were made on adult male and female albino rabbits weighing 3.0 to 4.0 kilograms. Pupillary diameters were measured with a clear plastic ruler under conditions of uniform illumination. Vertical and horizontal meridians were measured and the average values recorded. The intraocular pressure and intraocular pulse were measured by the Langham pneumatic tonometer with the floating tip sensor. Oxygen was supplied to the tonometer at a pressure of 20 pounds per square inch, and the sensor pressure was measured with a Sanborn 267B pressure transducer connected to a Sanborn 296 recorder. The tonometer was standardized each day against a pressurized Silastic membrane and the readings interpreted from a mean manometric calibration curve for rabbit eyes.

Tonometry was performed following application of 1 drop of 0.5 per cent proparacaine (Ophthalmic; Squibb and Sons, Princeton, N. J.). To reduce the duration of corneal anesthesia, the excess proparacaine was rinsed with 5 drops of physiologic saline after a few seconds.

**Adrenergic denervation.** Extirpation of the superior cervical ganglion was carried out by the method of Langham and Taylor through a 5 cm. vertical midline incision after intravenous-induced anesthesia with 1 ml. of pentobarbital supplemented with methoxyflurane by inhalation. The ganglion was located inferomedially to the carotid bifurcation and was confirmed by observation of unilateral pupil dilatation following electrical stimulation of the preganglionic segment. Preganglionic sympathectomies were made by excising a 2 cm. segment of the cervical sympathetic nerve. The wound was closed with sutures and tetracycline ointment and intramuscular procaine penicillin administered prophylactically. Intraocular pressure was measured at regular intervals following the procedure, and clonidine experiments were made no sooner than 2 weeks after surgery. At this time, the intraocular pressure on the side of the operation had recovered to values slightly below its original pressure and a few millimeters of mercury less than the intraocular pressure of the opposite eye. Adrenergic supersensitivity could be demonstrated at that time by a 4 to 5 mm. pupillary dilatation in the eye on the operated side following instillation of 25 μl of 0.1 per cent norepinephrine.

**Experimental procedure.** Solutions of experimental drugs were applied topically with a 50 μl automatic pipette (Eppendorf, Germany) or injected into a marginal ear vein. With topical application, care was taken to allow spread over the entire surface of the cornea. Intracocular pressure and pupil measurements were repeated at 30 min. and 1, 3, 5, and 24 hr. following administration of drugs.

**Preparation of drugs.** The following drugs were used: clonidine HCl (Glaucomes, 0.25 per cent and powdered base; Boehringer Ingelheim, Elmsford, N. Y.) and phenoxybenzamine (Dibenzyline; SmithKline Corp., Philadelphia, Pa.).

**Statistical evaluation.** All results are expressed statistically as the arithmetic mean ± standard error of the mean, and the number of results given in parentheses. Student's t test was used to determine whether the difference in means before and after treatment was significant.

**Results**

The mean time courses of the pupillary and pressure responses of six conscious rabbits to 50 μl of 0.25 per cent clonidine applied topically are shown in Fig. 1. A small but significant pupil dilatation developed in all the treated eyes within 15 min., with a mean mydriasis of 1.1 ± 0.4 mm. (p < 0.01) based on difference in pairs of eyes recorded at 1 hr. Recovery was complete within 3 hr.
Fig. 1. The mean intraocular pressure and pupillary responses of a group of six conscious rabbits to clonidine. 50 µl of 0.25 per cent solution of the drug was applied at zero time to one eye ( • • ). The contralateral eyes ( ○ ○ ) were not treated.

The time courses of the pressure responses in the treated and untreated eyes differed qualitatively. In the treated eyes, the pressure response was biphasic with an early hypertensive phase preceding a more prolonged hypotensive phase. In the untreated eye, there was a monotonic decrease of intraocular pressure. The mean pressure increment in the treated eyes over the first hour was 2.5 ± 0.9 (6) mm. Hg, and in the untreated eyes the mean pressure decrement was 3.5 ± 0.7 (6) mm. Hg. In individual rabbits, the mean intraocular pressure in the treated eyes at 1 hr. exceeded that in the untreated eyes by 5.5 ± 0.3 (6) mm. Hg. After the first hour, the intraocular pressure in the treated eyes fell to values similar to those of the untreated eyes. After the third hour, the intraocular pressures in both eyes increased and recovery was complete by approximately 5 hr. The time courses of the transient pupillary dilatation and ocular hypertension were similar.

Clonidine concentrations of 0.15 and 0.06 per cent applied topically induced qualitatively similar results. In the treated eyes, a transient hypertensive response developed, whereas a decrease of intraocular pressure developed in the contralateral eyes. By the end of the third hour, the intraocular pressures of the treated eyes had decreased to values similar to those of the untreated eyes.

The biphasic pressure response to clonidine applied topically was not seen when the drug was given intravenously (25 µg per kilogram). Fig. 2 shows the mean time courses of the pupillary and pressure responses. A significant fall of intraocular pressure was recorded at 5 min., with maximal bilateral pressure decrease of 8 to 10 mm. Hg present within 1 to 2 hr.; the intraocular pressure showed 50 per cent
recovery by 5 hr. The pupils demonstrated an immediate bilateral 1 mm. miosis that remained unchanged for 5 hr.

The administration of the α-adrenergic receptor antagonist, phenoxybenzamine, was found to inhibit the pupillary and hypertensive response but not the hypotensive response. In untreated rabbits, phenoxybenzamine given intravenously (5 mg. per kilogram) caused decreased intraocular pressure which slowly recovers in 6 to 24 hr. Four rabbits were treated unilaterally 90 min. after the intravenous injection of phenoxybenzamine (5 mg. per kilogram). No hypertensive or significant pupil dilation occurred, whereas a new bilateral decrease in intraocular pressure of 2.2 ± 0.3 mm. Hg was seen at 2 hr. In control rabbits given the same intravenous dose of phenoxybenzamine, the intraocular pressure showed a small increase over the same period. The mean intraocular pressures prior to and 90 min. after phenoxybenzamine administration were 20.5 ± 0.3 and 14.5 ± 0.4 mm. Hg, and two hours later the mean pressure was 15.7 ± 0.6 mm. Hg.

The influence of unilateral preganglionic cervical sympathectomy on the ocular response to topically applied clonidine was examined in six rabbits 3 weeks after surgery. At this time, there was a small but significant pressure decrease of 1.5 ± 0.2 (6) mm. Hg in the operated eyes. Application of clonidine to the innervated eye caused an initial hypertensive response followed by a hypotensive phase (Fig. 3, A). In the denervated eye, there was no initial rapid decrease of intraocular pressure so characteristic of the ocular response in innervated eyes (Fig. 3, A). In the same...
Fig. 3. The influence of cervical preganglionic sympathotomy on the ocular response to clonidine. The left hand figures show the mean responses of six conscious rabbits to 50 \( \mu l \) of 0.25 per cent clonidine applied to the innervated eyes (••). The right hand figures show the mean responses of the same rabbits to 50 \( \mu l \) of 0.25 per cent clonidine applied to the denervated eyes (•—•). The contralateral eyes (O—O) were not treated.

group of rabbits, application of clonidine to the denervated eyes caused an initial hypertensive response and a rapid decrease of intraocular pressure in the contralateral innervated eyes (Fig. 3, B). The intraocular pressures in both eyes remained below normal for more than 24 hr.

Adrenergic denervation supersensitivity in the ocular tissues of rabbits develops after excision of the superior cervical ganglion but not after preganglionic sympathectomy.\textsuperscript{21, 22} In a final series of experiments, the effect of adrenergic denervation supersensitivity on the ocular response to clonidine was examined. In six rabbits 3 weeks after unilateral excision of the ganglion, the pupillary and pressure responses to norepinephrine in the eyes on the operated side were 300 to 500 times more sensitive than in the contralateral eyes. At this time, the mean intraocular pressure of the operated eyes was 2.9 ± 0.5 mm. Hg below that of the innervated eye. There was also a small degree of miosis in the operated eyes. When 50 \( \mu l \) of 0.25 per cent clonidine was applied to the innervated eyes, a biphasic pressure response developed, qualitatively and quantitatively similar to that seen in unoperated rabbits. The hypotensive response, however, appeared to be more prolonged in that recovery was not complete at 24 hr. In the same animals, the intraocular pressures of the denervated eyes did not decrease significantly over
the first hour, a response always seen in the innervated eyes. One week later, the eyes were completely recovered, and the same dose of clonidine was applied to the denervated eyes. The pupil dilated at a rate and to an extent similar to that seen in normal eyes; there was no evidence of supersensitivity of the denervated iris to clonidine. The intraocular pressure showed an initial hypertensive response of 5.5 ± 0.3 mm Hg and then fell back to values similar to the initial value. In the contralateral innervated eyes, a rapid decrease of intraocular pressure developed and persisted for more than 5 hr.

Discussion

In these studies, clonidine has been shown to cause a biphasic intraocular pressure response, a transient period of increased pressure preceding a more prolonged period of decreased pressure. The ocular hypertensive phase was limited to the treated eye, whereas the hypotensive response developed in both the untreated and treated eyes. The hypertensive but not the hypotensive response was blocked by the α-adrenergic receptor antagonist, phenoxybenzamine, and it was shown that the hypotensive response was dependent on an intact adrenergic innervation of the eye.

There are good reasons for concluding that the initial ocular hypertensive response to clonidine is due to a local sympathomimetic action. The topical application of clonidine caused a small but significant pupil dilatation, and both this and the hypertensive response were aborted by prior intravenous administration of phenoxybenzamine. The ability of clonidine to stimulate α-adrenergic receptors in the eye, however, is weak compared with that of the physiologic catecholamines, and in this respect the receptor-agonist activity of clonidine was not significantly enhanced following adrenergic denervation of the eye. By way of comparison, the sensitivity of pupillary response to norepinephrine increased by over 2 log units in the same animals.

The evidence that clonidine has α-adrenergic receptor agonist activity is in agreement with the recent observations of Bill and Heilmann that clonidine causes a vasoconstriction of ocular vessels. Using radioactive-labeled microspheres to measure regional blood flow, Bill and Heilmann reported that the unilateral carotid injection of clonidine decreased the ipsilateral choroidal blood flow by 19 per cent and the blood flow to the iris and ciliary processes by 25 to 30 per cent.

A similar direct α-adrenergic receptor response to clonidine has been found in the systemic vascular response to clonidine. The intravenous injection of clonidine caused a biphasic change in arterial blood pressure, the prolonged hypotension being preceded by a transient increase of blood pressure. The initial hypertensive response, but not the hypotensive response, was blocked by prior administration of phenoxybenzamine. The initial systemic hypertensive response was of much shorter duration than the ocular hypertension seen in the present studies, but this discrepancy may be attributed to the rapid development of the vascular hypotensive response mediated by stimulation of receptors in the central nervous system following intravenous injection of the drug.

The qualitative characteristics of the hypotensive response differed strikingly from the initial hypertensive response in several ways. First, it was a bilateral response with a pressure decrease in both the treated and untreated eyes. Second, the full hypotensive response was dependent on an intact adrenergic innervation of the treated and untreated eyes, whereas the hypertensive response occurred in either the absence or the presence of adrenergic innervation. Third, the hypotensive response was not blocked by the intravenous injection of α-adrenergic receptor antagonists.

The ability of intravenous phenoxybenzamine to block the hypertensive and pupillary dilatation but not the hypotensive response to clonidine is strongly suggestive that the mechanism of the ocular hypotensive response is dependent on the central
nervous system activity of clonidine. This explanation is consistent with the observation that the hypotensive response was dependent on an intact adrenergic innervation and with the bilateral character of the hypotensive response.

The central action of clonidine in decreasing arterial blood pressure of animals and man is known to involve $\alpha$-adrenergic receptors, resulting in decreased sympathetic outflow to the heart and other organs.\textsuperscript{10-14, 20} The present observation that adrenergic innervation of the eye is essential for the ocular hypotensive response to clonidine suggests that the arterial and ocular hypotensive responses involve the same adrenergic mechanisms. The interesting question arises of how a decrease of adrenergic tone might cause decreased intraocular pressure. The problem is complex, not only because of our limited understanding of the neurophysiology of the cervical sympathetic nerve with its fibers of different conduction velocity and function,\textsuperscript{21, 22} but also because there is good experimental evidence that both a loss and an increase of adrenergic tone may decrease intraocular pressure.

Adrenergic denervation of the eye increases the ocular blood flow, and this may be accompanied by either short or prolonged decreases of intraocular pressure in animals\textsuperscript{22} and man.\textsuperscript{20} On the other hand, increase of adrenergic neural activity, e.g., that induced by electrical stimulation of the preganglionic cervical sympathetic nerve, also decreases intraocular pressure and ocular blood flow.\textsuperscript{24}

A clue to the cause of the hypotensive response may be found in observations that clonidine applied topically in man decreases intraocular pressure by reduction in the rate of aqueous humor formation.\textsuperscript{4, 5} The effect is very pronounced in both normal and glaucomatous eyes and is similar to the action of vasodilator drugs, including isoproterenol and the highly specific $\beta_2$ agonist, salbutamol, in decreasing intraocular pressure and the rate of aqueous humor formation.\textsuperscript{27, 28} The vasodilatation of the uveal vessels has been found to be accompanied by decrease of blood flow to the ciliary processes, a paradoxical response which has been attributed to a siphoning or shunting of blood into alternate pathways.\textsuperscript{29}

The possibility that the ocular hypotensive response to clonidine is partially due to a decrease of arterial blood pressure is valid, but it would appear to be of minor significance. A decrease of arterial blood pressure to the eye causes a disproportionately small effect on intraocular pressure. Unilateral ligation of the common carotid artery of rabbits decreases the ophthalmic blood pressure 30 to 40 mm. Hg and reduces the intraocular pressure by 2 to 4 mm. Hg.\textsuperscript{30} Following the intravenous injection of clonidine to rabbits, Sayegh and Weigelin\textsuperscript{17} reported that the arterial blood pressure of rabbits decreased from an initial mean value of 85 mm. Hg to 75 mm. Hg over a period of 1 hr. Over the same period, we found a similar decrease of blood pressure in conscious rabbits and a decrease in ocular pressure of 8 to 12 mm. Hg. Thus, although clonidine given intravenously or topically causes decreased arterial pressure, it is evident that the decrease in intraocular pressure cannot be explained by blood pressure decrease alone.

The responses to clonidine in rabbits differed from those in normal and glaucomatous eyes in two respects. First, clonidine applied topically to man does not induce an initial hypertensive response, and at 1 hr. a significant hypotensive response is already present. Second, the ocular hypotensive response to clonidine in man may be limited to the treated eye. This contrasts with the rabbit experiments where the hypotensive response in the treated eye was always accompanied by a similar hypotensive response in the untreated eye. The first finding is analogous to recent observations that $\alpha$-adrenergic agonists including epinephrine readily give an ocular hypertensive response in rabbits, whereas the response is less marked in man.\textsuperscript{31} The second finding may be explained by the fact that similar amounts of clonidine were ap-
plied to both rabbits and man, and in consequence the amount and concentration of drug absorbed systemically is most probably greater in the rabbit.

REFERENCES


