Vasoconstriction produced
in
the iris–ciliary body of the cat eye
by stimulation of local ganglion-like receptors

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The presence of local ganglion-like receptors in the anterior segment of the cat eye was demonstrated in an isolated, arterially perfused preparation. The administration of the known ganglionic stimulants, acetylcholine (Ach) plus eserine, pilocarpine, and dimethylphenylpiperazinium bromide (DMPP) produced a vasoconstriction. The actions of these stimulants were abolished by α-adrenergic blockade. Hexamethonium completely blocked the response to DMPP, was always ineffective as a blocking agent against Ach plus eserine, and sometimes abolished the response of pilocarpine. High concentrations of atropine always blocked the response to the three stimulant drugs. The data presented are also compatible with the currently accepted hypothesis of dual excitatory receptor sites on sympathetic ganglia.

Key words: iris, ciliary body, ganglia, vasoconstriction, pilocarpine, acetylcholine, eserine, norepinephrine, epinephrine, isoproterenol, dimethylphenylpiperazinium, hexamethonium, tolazoline, dibenzyline

It has been assumed that locally applied autonomic drugs which act on intraocular muscles and blood vessels do so by action on postganglionic neuroreceptors. The reason for this assumption has been that the principal sympathetic and parasympathetic ganglia which innervate the eye are located extracellularly. Although cells with a staining characteristic suggestive of ganglia have been demonstrated in the eye, their role in the mediation of pharmacologic responses has generally been ignored.

This report is made to demonstrate the activation of local ganglion-like receptors which affect blood vessels in the anterior uvea of the cat eye. Pharmacologic stimulation of these ganglion-like receptors produces a vasoconstriction of the iris–ciliary body blood vessels.

Methods
The procedure for the isolation and arterial perfusion of the cat iris–ciliary body has been reported. It consists essentially of perfusing the
preparation, under constant pressure, through an intrascleral portion of one of the two long posterior ciliary arteries. Eagle’s Basal Medium No. 2 is used as the perfusate fluid and bath. The iris-ciliary body preparation and perfusate are maintained at 36°C. Perfusate flow rate is monitored by a differential pressure-type flowmeter placed between the perfusate reservoir and the preparation. Drugs (calculated as base) are administered in fixed concentrations by turning stopcocks which allow the perfusate to be drawn from any one of a series of bottles in which they are contained. Using fluorescein, a lag time of approximately 20 seconds was found for the movement of fluid from the stopcock to the artery.

Results

One hundred and twenty-eight iris-ciliary body preparations from 64 cats were utilized for this study. The magnitude of vasoconstriction produced by the agonists used in these experiments was difficult to quantitate for comparative purposes. The criterion for alteration of vascular resistance is a change in the rate of constant pressure arterial perfusate flow. Two factors, however, made it unwise to select percentage change of flow rate as an indicator of drug activity. The first of these was a sometime continuous increase in responsiveness of the preparation to administer agonists during the entire experimental period. Second, there was no way of determining, in each case, what portion of the total perfusate flow passed through the reactive vascular bed in order to make percentage changes meaningful. These difficulties therefore dictated against our making quantitative dose-response comparisons of drug activity between different preparations. In all of the experiments reported here, only one agonist and one antagonist were studied on a single preparation.

Norepinephrine, acetylcholine (Ach) plus eserine, and pilocarpine, each in a concentration of 10 μg per milliliter, and dimethylphenylpiperazinium bromide (D MPP), 1 μg per milliliter, produced a vasoconstriction of the iris-ciliary body blood vessels. In all instances the vasoconstriction could be abolished by the simultaneous administration of tolazoline (10 μg per milliliter) (Figs. 1 to 4) or by a prior 15 minute perfusion with dibenzyline (10 μg per milliliter) (Fig. 5). Atropine, 10 μg per milliliter, administered concomitantly with each of the agonists, with the exception of norepinephrine, always abolished the vasoconstriction responses (Figs. 6 to 9). Hexamethonium (C-6) in a concentration of 10 μg per milliliter was found to block the vasoconstrictor action of D MPP in all cases (Fig. 10). In four of ten experiments, C-6 blocked the action of pilocarpine (Fig. 11) but never was seen to influence the response of Ach plus eserine (Fig. 12) or of norepinephrine (Fig. 13). Angiotensin, 0.1 μg per milliliter, was effective in producing a vasoconstriction in 8 of 8 iris-ciliary body preparations. Tolazoline, 10 μg per milliliter, when administered concurrently with the angiotensin did not depress the vasoconstrictor response. In order to be certain that the vascular α-adrenergic receptors in these preparations had been blocked, tolazoline alone was perfused through the preparation for ten minutes and a challenging trial of angiotensin then readministered (Fig. 14), but tolazoline still remained completely ineffective in blocking the angiotensin response.

Pupillary changes were observed grossly. The pupils during perfusion were miotic, and where the pupil was sufficiently open it could be seen to constrict further with the administration of Ach plus eserine and pilocarpine. Norepinephrine produced a marked mydriasis, D MPP a small mydriasis of 1 to 2 mm., and no pupillary response was noted with angiotensin.

Discussion

Tolazoline or dibenzyline in all experiments inhibited the vasoconstrictor response of the iris-ciliary body produced by either pilocarpine or Ach plus eserine. This is strong evidence that the final site of the produced stimulus is on α-adrenergic receptors and not on parasympathetic postganglionic neuroreceptors. The specific ad-
Figs. 1 to 4. 1, Perfusate flow rate through an isolated cat iris-ciliary body preparation. The vasoconstriction produced by l-norepinephrine is completely abolished by tolazoline. Time between heavy vertical bars = 20 seconds. 2, Decrease in arterial perfusate flow rate through the isolated iris-ciliary body of the cat produced by Ach plus eserine and blocked tolazoline. The decrease in perfusate flow is interpreted as vasoconstriction. Time between heavy vertical lines = 20 seconds. 3, Decrease in arterial perfusate flow rate through the isolated iris-ciliary body of the cat produced by pilocarpine and blocked by tolazoline. Time between heavy vertical lines = 20 seconds. 4, Decrease in arterial perfusate flow rate through the isolated iris-ciliary body of the cat produced by DMPP, 10 µg per milliliter, and blocked by tolazoline, 10 µg per milliliter. Time between heavy vertical lines = 20 seconds.
the same. Pilocarpine, in addition to its muscarinic action, is known to stimulate the parasympathetic agents were preferred to as "LN,"15 Category II,19 and E-2.20 The latter sites are activated by preganglionic stimulation. These secondary receptors have been referred to as "LN,"18 Category I,19 and E-2.20 The latter sites are activated by preganglionic nerve stimulation. C-6 was found to be completely devoid of blocking action when used against Ach plus eserine in the iris-ciliary body and produced no blockade in six of ten experiments with pilocarpine. Utilizing the cited criteria, Ach plus eserine may be said to stimulate only secondary ganglionic receptors of the iris-ciliary body, while pilocarpine could stimulate both.

reregangnic nature of the response is indicated by the ability of the blocking agents to inhibit the vasoconstriction of norepinephrine but to be completely ineffective in antagonizing the response of angiotensin. The possibility that the vasoconstrictor responses reported here are caused by iris or ciliary muscle movement seem remote, since the pupillary effects of the sympathetic and parasympathetic agents were opposite while the vascular responses were the same. Pilocarpine, in addition to its muscarinic action, is known to stimulate sympathetic ganglia,7-12 as do Ach13-16 and eserine.17 The high level concentrations (10 μg per milliliter) of atropine used in these experiments completely blocked the iris-ciliary body vascular response to either pilocarpine or Ach plus eserine. These data are compatible with findings obtained using the superior cervical ganglia in which much lower concentrations of atropine were found to inhibit the ganglionic stimulatory actions of pilocarpine15-17,18 and of Ach15-16. The ability of low concentrations of atropine, but not of C-6, to block the responses of the cervical sympathetic ganglia has been used as criteria to indicate that the stimulus site in the ganglia is on receptors other than those primarily activated by preganglionic stimulation. C-6 was found to be completely devoid of blocking action when used against Ach plus eserine in the iris-ciliary body and produced no blockade in six of ten experiments with pilocarpine. Utilizing the cited criteria, Ach plus eserine may be said to stimulate only secondary ganglionic receptors of the iris-ciliary body, while pilocarpine could stimulate both.
Fig. 8. Decrease in arterial perfusate flow rate through the isolated iris-ciliary body of the cat produced by pilocarpine and blocked by atropine. Time between heavy vertical lines = 20 seconds.

Fig. 9. Decrease in arterial perfusate flow rate through the isolated iris-ciliary body of the cat produced by DMPP, 10 μg per milliliter, and blocked by atropine, 10 μg per milliliter. Time between heavy vertical lines = 20 seconds.

Fig. 10. Decrease in arterial perfusate flow rate through the isolated iris-ciliary body of the cat produced by DMPP, 10 μg per milliliter, blocked by hexamethonium (C-6). Time between heavy vertical lines = 20 seconds.
Fig. 11. Decrease in arterial perfusate flow rate through the isolated iris-ciliary body of the cat produced by pilocarpine and blocked by hexamethonium (C-6). This blockade occurred in only four of ten preparations. Time between heavy vertical lines = 20 seconds.

Fig. 12. Decrease in arterial perfusate flow rate through the isolated iris-ciliary body of the cat produced by Ach-esterine which is unaffected by hexamethonium. Time between heavy vertical lines = 20 seconds.

Fig. 13. Per fusate flow rate through an isolated cat iris-ciliary body preparation. Hexamethonium (C-6) is completely ineffective in preventing vasoconstrictor action of L-norepinephrine. Time between heavy vertical bars = 20 seconds.

Fig. 14. The ineffectiveness of tolazoline to inhibit the vasoconstrictor response of angiotensin. After challenge with angiotensin in left plate, response was reversed with control perfusate solution. Tolazoline was perfused through the preparation for ten minutes prior to the administration of both agents simultaneously in right plate.
In order to strengthen the evidence for the presence of local ganglion-like receptors which influence the vasculature of the iris-ciliary body, DMPP was utilized. This compound has been reported to be a specific ganglionic stimulant, the action of which can be abolished by a classic ganglionic blocking agent, such as C-6. Perfusion of DMPP through the vasculature of the organ studied here produced a vasoconstriction which was indistinguishable from that produced by the cholinomimetic agents. As with the cholinomimetics, the response was abolished by the α-adrenergic blocking agents used to demonstrate the similar sympathomimetic nature of the response. In all instances, the vasoconstrictor response to DMPP was also abolished by C-6.

The evidence presented appears to be conclusive that ganglia or ganglion-like receptors are located within the tissues of the iris-ciliary body preparation and that these ganglia, when stimulated, can produce a vasoconstriction by an α-adrenergic mechanism.

The anatomic presence of uveal ganglia has been demonstrated by histologic technique, and the presence of accessory episcleral ganglia is generally recognized. The work presented here gives no indication as to the location of the ganglion-like receptors which were stimulated in these experiments. They may be the episcleral ganglia which are contained within the narrow segment of sclera which longitudinally surrounds the cannulated long posterior ciliary artery. This matter is being studied and will be the subject of a further report.

Conclusion

The presence of local, sympathetic, ganglion-like receptors which affect the vasculature of the anterior uvea of the cat eye has been demonstrated. These receptors have dual sites for excitation similar to those reported for the superior cervical ganglion.

REFERENCES


