Adrenergic nerves to the eye and to related structures in man and in the cynomolgus monkey (Macaca irus)

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The number of adrenergic nerves in the primate eye (Homo and cynomolgus monkey) is generally less than that seen in many lower mammals, as revealed by the fluorescence technique of Falck and Hillarp. Adrenergic nerves were found, in various numbers, to reach the limbus corneae, scleral aqueous drainage channels, dilator pupillae, sphincter pupillae, ciliary muscle, ciliary part of the uveal meshwork of the chamber angle, choroid, lacrimal gland, tarsal muscle, and smooth muscles of the gland of Meibom. In the ciliary ganglion, monoamine-containing neurons can be found under certain conditions. The perivascular sheath of adrenergic nerves has a peculiar spacious arrangement in the iris, similar to that found in the iris of other mammals. In some of the iridic vessels, adrenergic fibers are found throughout the smooth musculature. Varicose adrenergic nerves can be found to a considerable extent in nerve trunks as they approach their target organs. The retina contains a layer of adrenergic fibers between the inner nuclear and plexiform layers. Adrenergic cell bodies are found in the inner nuclear layer and, to a lesser extent, in the ganglion cell layer. No adrenergic fibers or cells were identified in the scleral trabeculae, at the canal of Schlemm, in the sclera proper, or in the optic nerve.

Great anatomical differences in the morphology of the chamber angle of primates and lower mammals have long been recognized. However, there are no well-documented comparative studies on the distribution of either sympathetic or parasympathetic fibers. Nevertheless, many of the conclusions regarding the physiology and pharmacology of the sympathetic nerves of the human eye have been extrapolated from studies on lower mammals. The recently demonstrated species differences in the distribution of adrenergic nerves to the eye in many lower mammals have necessitated further studies in order to obtain morphological foundations for neurofunctional comparisons between primates and lower animals. For this purpose, the sensitive and highly specific fluorescence method of Falck and Hillarp for the demonstration of certain monoamines has been used in this investigation of the innervation of the primate eye.

Materials and methods

Seventeen human eyes were obtained from operative specimens following enucleation for vari-
ous reasons: tumors 9, trauma 3, chronic uveitis 3, and chronic congestive glaucoma 2. Eyes were also obtained from 22 young cynomolgus monkeys, Macaca irus. Specimens were freeze-dried, treated with formaldehyde gas, and sectioned in various planes for fluorescence microscopy for the demonstration of adrenergic nerves according to Falck and Hillarp. Optimal visualization of adrenergic fibers was secured by the use of paraformaldehyde batches stored at different but constant relative humidities. Generally, the eyes were kept in dry ice for a few days before processing. Approximately 500 sections from each eye have been studied. In order to prevent the sections from coming off the slides during the various treatments, they were covered by a thin layer of celloidin, which was found not to affect the specific fluorescence. Reserpinization was effected in one monkey by the intravitreal injection of 50 μg Serpasil,* 24 hours before the enucleation.

The slides were examined in dark-field illumination as previously described. Certain slides were photographed using activating filters that gave some nonspecific tissue fluorescence for the demonstration of tissues surrounding the nerve fibers. Mast cells were identified by comparing sections stained with alcoholic toluidine blue with previously taken fluorescence micrographs.

Results

Judged by the histochemical and pharmacological criteria, all the structures described here as adrenergic contained a primary catecholamine. After the reserpinization, no adrenergic structures remained visible. Tissues processed without the formaldehyde gas do not develop any specific fluorescence, but sometimes show a considerable autofluorescence, and thus must constantly be used for reference. The autofluorescence varies between individuals (usually being lowest in young ones) but not within one and the same subject. In order to differentiate specific fluorescence from nonspecific, it is generally sufficient to use only untreated segments from the same eye, especially as the nonspecific fluorescence is slightly yellower than the specific. In doubtful cases, however, the reduction method of Corrodi and associates was used (3 minutes in 98 per cent 2-propanol with approximately 0.02 per cent sodium borohydride).

In the human material, it was not possible to verify all details in each eye because only parts without signs of pathological changes were used. Unless otherwise stated, the following descriptions refer to both species, human and monkey, although they are founded mainly on studies on monkeys. Only minor differences are obvious between the two species. On the whole, the two primates have distinctly fewer adrenergic nerves in the eye than many lower mammals, a feature which is even more pronounced in humans than in monkeys.

Cornea. In the cornea, no adrenergic fibers have been found, but very fine ones could hide behind the autofluorescence of this tissue. The limbus has a scant network of varicose adrenergic fibers throughout the superficial stromal layers. The media of the vessels is surrounded by varicose adrenergic fibers in the ordinary way. There are no nerve trunks that contain fluorescent fibers running toward the cornea as are seen in many lower mammals.

Sclera. In the sclera, the high autofluorescence makes exact studies difficult, but so far no adrenergic nerves have been found except in connection with penetrating vessels or nerve bundles. No adrenergic varicose fibers have been observed at the canal of Schlemm, although some fibers occur farther outward along the aqueous drainage channels.

Iridocorneal angle. In the iridocorneal angle (for anatomical terminology, see Rohen), the scleral trabeculae (in front of the scleral spur) have a particularly strong yellowish autofluorescence (Fig. 1) with a distribution equivalent to that of structures stainable with orcein or other stains believed to have a specific affinity for elastic fibers. Fluorescent axons might be obscured by this autofluorescence, but extensive application of the sodium borohydride reduction and other tests has not supported any assumption of adrenergic nerves in this region (Fig. 1). A wide-meshed plexus of mainly meridionally directed varicose adrenergic fibers is found in the ciliary...
part of the uveal meshwork. This network merges with that surrounding the major iridic artery. The latter network is of an unusually loose and spacious type compared with the more compact network of adrenergic nerves surrounding arteries elsewhere in the body.9,11

Ciliary body. The ciliary body has a moderately dense subepithelial plexus of varicose adrenergic fibers, situated partly around the vessels, but also to a large extent in a separate plexus between the vessels and the ciliary epithelium. This organization can also be followed into the ciliary processes (Fig. 2). However, the distinction between vascular and nonvascular adrenergic fibers is less evident in these than in the ciliary body. There are no intraepithelial fibers. Often, a yellowish, fibrillar autofluorescence can be seen in the loose connective tissue under the epithelium.

Ciliary muscle. In the ciliary muscle, there is a moderate number of varicose adrenergic fibers running among and mainly parallel to the smooth muscle bundles (Fig. 3). The meshes are wide and seem to engulf several muscle cells. In some cases, the circular portion of the muscle seemed to have a somewhat looser network of adrenergic fibers than the other portions. The plexus is never so dense as that described by authors using methylene blue or metallic impregnation techniques.22 Connections between the adrenergic fibers and capillaries are not apparent. Occasionally, large nerve trunks are encountered, running meridionally in the ciliary body near the sclera and containing some varicose adrenergic fibers as well as myelinated nerve fibers. Due to the autofluorescence in the nerve bundles, it has not been possible to assess the amount of preterminal smooth adrenergic fibers in them.

Cells with a weak, greenish fluorescence of varying intensity can be seen very sparsely distributed in the ciliary body of the monkey. Their morphology is equivalent to that of the nerve cells found with classical staining techniques.13 Some of the mast cells show a yellowish, granular fluorescence, the intensity of which varies from very strong to weak. Their number varies from animal to animal. They bleach.
Fig. 2. Ciliary process, cynomolgus monkey. At the arrow, a yellowish mast cell. The fibrillar fluorescence comes from adrenergic nerves, partly lying at vessels. The epithelium has a diffuse, greenish autofluorescence. (x160.)

on borohydride reduction, although more sluggishly than the adrenergic nerves. The melanophores in the ciliary body are of a nonfluorescent type without any observable connection to adrenergic nerves.

Iris. In the iris, the varicose adrenergic fibers lie both within the dilator muscle and (particularly in some of the human irides) immediately anterior to it. The sphincter pupillae contains some varicose adrenergic fibers, most of them appearing in the peripheral part of the muscle (Fig. 4). Some have no detectable connections to vessels. The middle iridial layer contains only few adrenergic fibers. Nerve bundles containing varicose adrenergic fibers are not uncommon. Rarely, in short segments of some of the medium-sized iridic vessels, adrenergic fibers can be seen to reach through the media, coming close to the intima (Fig. 5). The fibers can be separated easily from the inner limiting membrane, which may show some autofluorescence, and they have the varicose appearance typical of nerve terminals.

In the frontal layer of the monkey iris, there is a moderately dense plexus of fine varicose adrenergic fibers running among and in close association with the star-shaped melanophores (Fig. 6). In the mon-

Fig. 3. Ciliary muscle, circular portion, cynomolgus monkey. The black, irregularly shaped structures are nonfluorescent melanophores. The adrenergic nerve fibers have the appearance of strings of beads. (x200.)
Fig. 4. Iris, sphincter region, cynomolgus monkey. The dilator pupillae is seen as a homogeneously fluorescent band, not quite extending to the pupillary margin. The sphincter lies in front of it, containing many fluorescent nerves in its peripheral part. The middle iridic stromal layer contains only few fluorescent fibers, whereas these can be seen to accumulate toward the front of the iris. (x95.)

Fig. 5. Medium-sized iris vessel with some adrenergic fibers reaching through the media. Cynomolgus monkey. (x340.)

key, these cells have a dark red autofluorescence. It does not fade upon ultraviolet irradiation in contrast to the red fluorescence of the murine Harderian gland, a fluorescence which is probably caused by porphyrins. The adrenergic fibers accumulate toward the front of the iris, as do the melanophores. Adrenergic nerve fibers in the human iridic stroma are scarcer than in the monkey, and it is not immediately evident in the human that every melanophore is in contact with an adrenergic fiber. However, the main distribution of the adrenergic fibers is similar to that of the monkey, and the star-shaped melanophores also accumulate toward the front. The human melanophores show no, or only weak, autofluorescence, in contrast to those of the monkey. The clump cells, which emit a weak, reddish-brown autofluorescence, do not seem to be connected to adrenergic nerves.

Retina. In the retina, a green-fluorescent, thin layer of varicose adrenergic fibers is found at the border between the inner nuclear and plexiform layers. It originates from adrenergic perikarya in the innermost cell rows of the inner nuclear layer (Fig. 7). Varicose adrenergic fibers issue from the layer of adrenergic fibers and penetrate some distance into the inner nuclear layer, often forming baskets of fibers around nonfluorescent cell bodies. Further, in the ganglion cell layer in the monkey, adrenergic perikarya can be seen with a frequency of approximately one in ten adrenergic cells of the inner nuclear layer. In many cases, a process can be seen to extend to the layer of adrenergic fibers at the inner nuclear layer (Fig. 8). Very few adrenergic fibers accompany the retinal vessels.
Fig. 6. Iris, anterior part near the sphincter, cynomolgus monkey. The peripheral part of the sphincter can be seen in the lower-right-hand corner. The spider-shaped melanophores with a red autofluorescence can be faintly seen, forming the main part of the background fluorescence. The varicose adrenergic fibers mainly lie in the frontal third of the iris. (x185.)

Fig. 7. Retina, cynomolgus monkey. To the right, phase contrast micrograph, to the left, the fluorescence picture of the same area. The layer of adrenergic fibers is seen to lie between the inner nuclear and plexiform layers. An adrenergic cell body is also seen. At the bottom of the picture is seen the autofluorescence of the membrane of Bruch and the pigment cells. (x270.)

**Chorioid.** In the chorioid, varicose adrenergic fibers form the ordinary sheath around the large vessels. There is also a network of such fibers without apparent connection with any vessels.

**Optic nerve.** In the optic nerve, no adrenergic fibers have been found, except around the central artery, which has relatively many varicose adrenergic fibers superimposed on the muscular coat, as is usually seen in peripheral vessels. cf. 24-11

The number of fibers is abruptly reduced in the optic papilla very soon after the passage through the cribriform plate.

**Ciliary ganglion.** In the ciliary ganglion (of the monkey), no structures show any specific fluorescence in the normal animal. However, after the intraperitoneal injection of the monoamineoxidase inhibitor, nialamide (100 mg. per kilogram 4 hours be-
Fig. 8. Retina, cynomolgus monkey. To the left, phase contrast micrograph, to the right, the fluorescence picture of the same area. An adrenergic cell of the ganglion cell layer can be seen, issuing a process toward the layer of adrenergic fibers. At the bottom of the picture is seen the autofluorescence of the membrane of Bruch and the pigment cells. (x350.)

Fig. 9. Lacrimal gland, cynomolgus monkey. Practically every acinus is reached by adrenergic fibers. (x105.)

fore death), followed by L-dopa (100 mg per kilogram, 1½ hours before death), about one-fifth to one-tenth of the total number of nerve cells show a green to yellowish-green fluorescence. The intensity of the fluorescence varies between the cells from moderate to very strong. Around a few of the nonfluorescent cells there appears a dense cocoon of varicose green to yellowish-green fibrillar structures.

Lacrimal gland. The lacrimal gland (of the monkey) has a fine network of varicose adrenergic fibers running in between the secretory acini (Fig. 9). In some of the acini, granules occur with an orange autofluorescence; in others, there are no such
granules. The nerve distribution is the same to all acini. Every secretory cell seems to lie in close connection with a varicose adrenergic fiber. These are never seen to penetrate into the acini but form a loose network around them, each nerve fiber evidently being in proximity to several exocrine cells. No specialized nerve endings have been observed and the nerves seem to form an autonomic ground-plexus in the sense of Hillarp. The secretory ducts appear to have no adrenergic nerves and, consequently, there is probably no adrenergic innervation to the myoepithelial cells of these ducts.

Eyelid. In the eyelid (of the monkey), the levator palpebrae has the same coarse-latticed nerve pattern as described in lower mammals. The tarsal plate has unexpectedly many varicose adrenergic fibers (Fig. 10), some of which follow blood vessels. Others seem to supply smooth muscle fibers associated with the intratarsal parts of the glands of Meibom. The sudoriferous glands (of Moll) have no adrenergic innervation. The vessels have the ordinary adrenergic nerve sheath.

Discussion

The histochemical reaction developed by Falck and Hillarp for demonstrating adrenergic structures is based on the condensation of formaldehyde with the biogenic monoamines (adrenalin, noradrenalin, dopamine, and 5-hydroxytryptamine) which yields highly fluorescent products. The chemistry of the reaction is well known. The procedure has been applied to numerous peripheral tissues as well as to central nervous tissues in a large variety of species, and its specificity and sensitivity, under proper precautions, are thoroughly established.

Adrenergic fibers have been demonstrated in the cornea of various laboratory animals, but it has not been possible in the present study to show any such fibers in the primate cornea. However, its autofluorescence might conceal a small number of fine fibers. Further, a few corneal adrenergic fibers have been observed in human embryos. Thus, it cannot be excluded that such fibers may also be present in the adult. However, there are no large nerve trunks containing several varicose adrenergic fibers, such as can be seen in lower animals.

In the trabecular meshwork, the autofluorescence may also disguise some fine adrenergic fibers, but any substantial number would be revealed by the borohydride reduction test (Fig. 1). Moreover, the auto-
fluorescence was quite low in a few animals; despite this, no adrenergic fibers were visible.

As only few adrenergic varicose fibers were seen to follow blood vessels in the sphincter pupillae, an adrenergic innervation may be attributed to this part. It is of interest to note in this connection that vessel injection experiments\(^2\) have clearly demonstrated that, in the rabbit, guinea pig, and rat, the majority of adrenergic nerves of the sphincter run separated from the vessels and, further, that in the cat there is a very rich supply of adrenergic fibers in the sphincter.\(^3\) This also strongly suggests an adrenergic innervation in these species.

The vessels of the iris are known to have a peculiar connective tissue sheath with a loose inner part and a stiffer outer layer. The varicose adrenergic fibers of the vessels are situated throughout the loose part and their unusual, spacious arrangement is probably due to the mobility and laxity of this layer. The rabbit and the cat have a similar system.\(^2, 3\) Further, there are normally no adrenergic fibers between the muscle cells of the blood vessel wall.\(^4-11\) However, exceptions to this rule have been found not only in the monkey iris. In some penile vessels, the media is richly supplied with adrenergic fibers.\(^21\) Adrenergic fibers reaching the intima have also been observed in iridic vessels in the rabbit\(^2\) and in some vessels in the fingertips of the monkey.\(^21\)

Founded on the clinical observation of heterochromia in Horner's syndrome and classical morphological studies,\(^2\) a sympathetic innervation has been ascribed to the iridic melanophores. The present morphological study lends some support to this conception as far as primates are concerned, but in lower mammals there is no evidence of such an innervation.\(^2-4, 24\)

Many varicose adrenergic fibers occur in nerve trunks of the primate eye as they approach their effector organs. These fibers have the same morphology and fluorescence characteristics as varicose terminals running among the effector cells. A similar distribution has also been found in the eyes of the cat, dog, and rabbit,\(^2-4\) in the male genital organs of various mammals,\(^23\) and in many other regions.\(^24\) Thus, this phenomenon seems to be the rule rather than the exception. Norberg and Hamberger\(^10\) state that varicosities of the adrenergic fiber occur only as synaptic structures and appear precisely at the effector site. The present findings agree with this statement only on the assumption that a release of the transmitter also occurs within the nerve trunk itself. On the other hand, it cannot be excluded at present that the varicosities represent a store where the transmitter is not immediately available for the transmission.

A sympathetic innervation has long been postulated to exist along with the well-established cholinergic innervation in the ciliary muscle.\(^22, 25\) More recently, van Alphen and associates\(^26\) demonstrated a relaxation of the monkey ciliary muscle upon the application of sympathomimetics. In the present study, the adrenergic fibers of the ciliary muscle were not seen to contact either blood vessels or melanophores. Thus, the adrenergic innervation of the primate ciliary muscle now seems well established. It should, however, be noted that in, e.g., the cat, no adrenergic fibers have been found in the ciliary muscle.\(^3\)

The layer of fluorescent neurons in the inner nuclear layer of the retina corresponds to that found in the rabbit and the rat. In the rabbit,\(^27, 28\) guinea pig, and rat\(^27\) additional adrenergic neurons may be found at certain levels in the inner plexiform layer, but there have been no signs of such fibers in the retina of the cynomolgus monkey. On the other hand, the adrenergic neurons in the ganglion cell layer of the monkey have counterparts in the cat, rabbit, guinea pig, and rat.\(^3, 4, 27\)

The lacrimal glands of the monkey possess an adrenergic innervation similar to that of the dog and the cat.\(^5, 4\) Some rodents, in contrast, have an exclusively cholinergic innervation to this gland.\(^5, 4\) In the monkey, every acinar cell seems to be sup-
plied with one or more adrenergic fibers which form a plexus similar to that found in some salivary glands, e.g., the submaxillary glands of the rat and the sublingual and submaxillary glands of the monkey.

In adrenergic nerves and nerve cells, efficient uptake and storage mechanisms for catecholamines have been demonstrated. The fluorescence observed in some of the nerve cells in the ciliary ganglion after the injection of nialamide and L-dopa indicates the presence of a similar mechanism, clearly distinguishing them from the others in the ganglion. Similar cells have been found in the ciliary ganglia of the rat and rabbit. They may be adrenergic, although this is not proved. The observed absence of fluorescent cells in ganglia from normal animals does not necessarily imply that the ganglion is devoid of adrenergic cells. Adrenergic neurons which have nonfluorescent cell bodies, but which issue fluorescent axons and develops fluorescence in the perikaryon upon the treatment with nialamide and L-dopa, have been observed in the peripheral ganglia of the rabbit vas deferens. Also, true adrenergic fluorescent cells have been observed in the ciliary ganglion in the young, normal dog. Obviously, the former concept of the ciliary ganglion as a purely cholinergic, parasympathetic structure must be reconsidered.

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REFERENCES


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