Editorials on Recent Advances

Adrenergic modulation of the outflow of aqueous humor

The influence of the adrenergic nervous system on the regulation of intraocular pressure has been explored by electrical stimulation of the cervical sympathetic nerve, by surgical and pharmacologic denervation of the sympathetic supply to the eye, and by the administration of exogenous adrenergic agonists and antagonists. New information about adrenergic influences upon the outflow of aqueous humor has resulted.

Evidence for adrenergic effects. In the vervet monkey, stimulation of the cervical sympathetic nerve results in a slight increase in the inflow of aqueous humor with either no change or a decrease in outflow. In the rabbit, nerve stimulation increases outflow slightly. This increase would undoubtedly be greater were it not for the very efficient process of inactivation of released norepinephrine through re-uptake and binding by the densely innervated iris. A greater increase in outflow occurs when re-uptake and binding is prevented by pretreatment with cocaine or after denervation. In the latter case, supersensitivity can be demonstrated when norepinephrine is introduced into the anterior chamber. After denervation, 10 ng. of norepinephrine (base), corresponding to $2.5 \times 10^{-7}$ M in the anterior chamber, produces an increased outflow of aqueous in the sympathetically denervated eye. In the normally innervated eye, $1 \times 10^{-5}$ M norepinephrine is required to produce any increase in outflow.

Linner and Prijot, using the technique of surgical denervation of the cervical sympathetic supply to the eye and studying the steady-state intraocular pressure, first demonstrated that 24 hours after cervical ganglionectomy in rabbits, an ipsilateral decrease in intraocular pressure occurs. Experiments utilizing perfusion indicate that increased outflow of aqueous humor, caused by a release of norepinephrine into the anterior chamber from degenerating nerve terminals in the iris, causes the decrease in intraocular pressure.

Detailed studies of degeneration release of neurotransmitter after cervical sympathetic ganglionectomy provided conclusive evidence for the presence of an alpha-adrenergic receptor for outflow. The increase in outflow after ganglionectomy is (1) largely prevented by prior elimination of norepinephrine stores with systemically administered reserpine, or alpha-methyl-meta-tyrosine, and completely prevented with alpha-methyl-para-tyrosine; (2) blocked by the prior administration of phentolamine, dibenamine, and dibenzylne;
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(3) preceded in its time course by a decrease in the endogenous catecholamine content of the iris; (4) related directly to the appearance of norepinephrine in the perfusate (aqueous humor of the anterior chamber); and (5) duplicated by substances causing release of norepinephrine, such as reserpine, or by close (intracameral) injection of norepinephrine. Apart from the conclusion that an alpha-receptor for outflow exists, other studies of the ganglionectomy model, using reserpine, showed that the magnitude of the increase in outflow is reduced by a parallel reduction in the size of the iridial pool of norepinephrine available for release after ganglionectomy. Similar reductions after iridectomy identified the iris as the source for norepinephrine.

The results of exogenous administration of adrenergic effectors, false transmitters, and blocking agents strengthen the argument for adrenergic participation in the regulation of the outflow of aqueous humor. Problems in the route of delivery of the drug, the need to disentangle vascular from steady-state effects on the formation of aqueous humor, and the necessity to account for true facility, pseudofacility, pressure-dependent outflow (trabecular), and pressure-independent outflow (uveoscleral) make conclusions from in vivo studies hazardous, particularly when the pharmacologic effects produced are small. Under carefully controlled conditions, however, it is apparent from experiments employing intracameral injections of adrenergic substances, that there is alpha- and beta-adrenergic reception for outflow from the rabbit eye, and beta-reception from the monkey eye. In man the appropriate tests remain to be done.

Effects of topical epinephrine. The effects upon the outflow of aqueous humor after adrenergic drugs applied topically to the human eye are complex, but probably there are three: (1) an early decrease in pressure which appears to be related both to a decrease in aqueous inflow and an increased outflow; (2) a second prolonged phase of increased outflow; (3) a late, progressive increase in outflow even days, weeks, and months after therapy.

Early effect. Minutes after administration of topical epinephrine, the eye first becomes white, alpha-adrenergic effects (vasoconstriction and mydriasis) predominate, and possibly a fall in ciliary blood flow occurs. Studies of fluorescein turnover in the human eye, after topical epinephrine, indicated that the initial reduction in intraocular pressure was a consequence of decreased inflow of aqueous. Tonographic studies done in the 1950's suggested that not all the reduction in intraocular pressure could be explained in this manner. Recent tonographic studies of this early effect showed an increased outflow shortly after a single topical dose of epinephrine, that could last hours longer. The early decrease is, in part, actually a pseudofacility, i.e., a decrease in the pressure-dependent part of aqueous inflow. (Tonography measures gross facility only.) The remainder of the increase in outflow is probably an increase in true facility of outflow, similar to the effect after degeneration release of norepinephrine.

If part of the increase in outflow is in reality a decrease in (the pressure sensitive part of) aqueous humor formation, how might this effect occur? The aqueous humor is formed by a process of ultrafiltration and secretion across the blood-aqueous barrier. In all likelihood these two processes are linked in series. The first step in the formation of aqueous humor is the development of a plasma filtrate in the stroma of the ciliary processes by "ultrafiltration" across the fenestrated capillary wall. The ciliary epithelia act upon this stromal pool. Although there are no nerves within the epithelial layers of the ciliary processes, alpha-adrenergic receptors are probably present in the vessels of the stroma to mediate vasoconstriction. Limiting the plasma filtrate entering the ciliary stroma by vasoconstriction, before it is acted upon by the epithelia, will decrease formation (inflow occurring through ultrafiltration)
and appear as an increase in total facility with manometric or tonographic techniques. A direct effect on true facility of outflow at this stage is probably also alpha-mediated because the eye still manifests other alpha effects: mydriasis and vasoconstriction, and there is extensive pharmacologic data to support alpha influences upon outflow (see above).

Intermediate effects. After topical epinephrine, an increase in outflow, intermediate in onset, almost certainly represents an increase in true facility. This second phase of increased outflow occurs and lasts many hours after topical epinephrine. Although an early alpha-mediated increase in true outflow facility may occur, hours after topical epinephrine, an increased outflow persists at a time when the eye is no longer white (from vasoconstriction) and the pupil no longer dilated. These intermediate effects, at least in part, involve the production of cyclic-AMP. In the rabbit, changes in cyclic-AMP in the aqueous humor, possibly a reflection of intracellular events, have been used to correlate the effect of adrenergic agents with changes of intraocular pressure. Topically administered epinephrine increases cyclic-AMP in the aqueous humor, the increase persists more than five hours, and it can be prevented by appropriate antagonists. Further, smaller decrements in intraocular pressure after epinephrine from extended daily treatment is accompanied by smaller increases in cyclic-AMP in the aqueous humor. On the other hand, after sympathetic denervation, an exaggerated decrease in intraocular pressure after epinephrine, is accompanied by an enhanced increase in the cyclic-AMP content of the aqueous humor. The increase in outflow facility and in cyclic-AMP production are both two to three times normal in the supersensitive eye. Finally, a two- to threefold increase in outflow is seen after direct intracameral injection of cyclic-AMP in a final molar concentration (anterior chamber) of $2 \times 10^{-4}$ M. Some analogs are even more potent. The likelihood that cyclic-AMP mediates the intermediate phase of increased outflow after epinephrine may prove a useful hypothesis for further investigation.

Beta-reception after topical epinephrine is probably responsible for the production of cyclic-AMP. What might be the receptor cell for the action of epinephrine or its cyclic nucleotide mediators? Major changes in the resistance to the outflow of aqueous humor through the conventional channels of outflow may be caused by the ciliary muscle acting on the meshwork or by direct drug action on the mesh or endothelial lining of Schlemm's canal. Although the idea has been advanced that dilation of the iris might transmit mechanical pull onto the trabecula, the increased outflow seen after cyclic-AMP (intermediate phase) occurs without mydriasis, making this idea unlikely. Pharmacologic analysis of the exaggerated increase in outflow produced by adrenergic agonists after ganglionectomy and after pharmacologic denervation with guanethidine and 6-hydroxydopamine (denervation supersensitivity) implies an autonomic effector with contractile properties. In most species, the endothelial cells of the meshwork and of the canal of Schlemm are only occasionally innervated; nevertheless, the cells lining the canal have mitochondria, indented nuclei, and cytoplasmic rodlike structures, all characteristics of cells with contractile properties. Adrenergic terminals found in the monkey and human chamber angle are, for the most part, located just anterior to the insertion of the longitudinal ciliary muscle. In the rabbit, such innervation is lacking or very difficult to find. Nonetheless, in the rabbit, in particular, there is a forward prolongation of ciliary muscle cells into the filtration angle. Although the ciliary muscle is sparse in the rabbit eye, these anatomic observations make it difficult to rule out an adrenergic outflow function for the ciliary muscle. Thus, current evidence cannot distinguish among the trabecular cells, endothelia of Schlemm's canal, or ciliary smooth muscle cells as receptor sites for adrenergic stimulation.
If a contractile mechanism in one of these tissues is responsible for an increase in outflow, what are its subcellular components? There is undoubtedly a membrane receptor for adrenergic agents, for example, exogenously applied epinephrine activates membrane-bound adenyl cyclase to increase the intracellular rate of production of cyclic nucleotide. In cells with contractile properties, a contraction-relaxation cycle may be influenced by cyclic-AMP levels, an increase in the level of cyclic-AMP causing (ATP and calcium-dependent) relaxation of smooth muscle. Muscle relaxation, associated with protein phosphorylation by a cyclic-AMP-dependent protein kinase, would be appropriate to the known beta-adrenergic functions of the ciliary muscle and produce the physiologic effect, increased outflow. This would not necessarily exclude effects on mesh endothelia. Thus the loci of the protein kinase and protein substrate for the cyclic-AMP system is still open to further speculation. It is clear that at these or a similar site, cyclic nucleotides can favor the outward movement of water from the eye and may do so by influencing movement in the cells regulating outflow or by changing their subcellular structure. Further studies are in order to decide how cyclic-AMP increases the outflow of aqueous humor but the concepts and tools are available for a study of the system.

Late effect. Finally, a late increase in outflow, that occurs over a period of weeks or even months with continued topical application of epinephrine, has been well characterized by Ballintine and supported by data gathered by other ophthalmologists. This late or cumulative effect of adrenergics on the outflow of aqueous humor is puzzling. It is known that the meshwork is coated with a mucinous material: in the rabbit, hyaluronidase-sensitive hyaluronic acid; in man, perhaps another mucin. Such coating provides resistance to outflow and may be affected by adrenergic compounds. Experiments done in the aorta of rabbits indicate that epinephrine may decrease the production of mucoid substances. Similar effects in the trabecular meshwork, eliminating resistance produced by a mucoid coat, could in time decrease the resistance to outflow of aqueous humor.

Glaucoma and adrenergic functions.
Finally, it should be stressed that these observations and speculations are intended to apply only to physiology and not to pathologic disturbances. The abundance of new basic and clinical information about the sympathetic nervous system can and should be organized into a useful frame of reference for application to diagnostic and therapeutic problems of a clinical nature. However, no evidence exists at present to link any of the varieties of open-angle glaucoma to any adrenergic dysfunction. Adrenergic compounds are useful agents in the therapy of glaucoma but the pathologic disturbance of the outflow channels in open-angle glaucoma is probably not a consequence of faulty adrenergic regulation.

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