Epinephrine Synthesis in the Rat Iris

Hamzeh Elayan,* Brian Kennedy, and Michael G. Ziegler

Epinephrine (E) alters blood flow, intraocular pressure and pupillary constriction. The rat iris contained E-forming activity that was moderately specific for a phenylethanolamine and was inhibited by the phenylethanolamine-N-methyltransferase (PNMT) inhibitor SKF 29661. Unilateral superior cervical ganglionectomy decreased iris norepinephrine (NE) 63%, but failed to lower PNMT activity or E in the iris. Removal of both adrenal medullae markedly lowered circulating E levels, but had no effect on iris E. Further treatment with 6-hydroxydopamine and reserpine greatly lowered iris NE levels, but failed to decrease either iris E or E forming activity. The rat iris has non-neuronal E-forming enzymes which appear to synthesize most of the E contained in the iris. Invest Ophthalmol Vis Sci 31:677-680, 1990

Epinephrine (E) has such important effects on the eye that both E and β-adrenergic blocking agents are common ophthalmic medications. Epinephrine stimulates the same α and β-1 receptors as norepinephrine (NE), but also stimulates β-2 receptors. The iris has a rich autonomic innervation and β receptors in the iris inhibit constriction of the sphincter muscle.1

It is generally thought that NE is made in noradrenergic neurons and E is synthesized in the adrenal medulla and to a small extent in the brain. However, Cohen and Hadjiconstantinou2 reported that E and its synthetic enzyme phenylethanolamine-N-methyltransferase (PNMT) were present in rat retina. Since adrenergic receptors alter blood flow and intraocular pressure, E synthesis by other structures in the eye could be quite important. We examined E synthesis in the rat iris, since the iris is relatively easy to study and has been the target of several prior studies of sympathetic neuronal influences.

Materials and Methods

All experiments were performed in accordance with the Arvo Resolution on the Use of Animals in Research. Four male Sprague-Dawley rats weighing 200-250 g underwent unilateral superior cervical ganglionectomy. Three days later, the rats were sacrificed by decapitation and iris, eyelid, facial skeletal muscle and salivary glands were removed from ipsilateral and contralateral (control) sides of each rat. Samples were washed with ice-cold saline and stored at -70°C. In another experiment, male Sprague-Dawley rats weighing 180-200 g were subjected to either bilateral adrenal demedullation (20 rats) or sham operation (nine rats) under ether anesthesia. All animal care and surgery were carried out according to the American Veterinary Medical Association recommendations for humane care of animals. Adrenal demedullation was performed by making an incision through the most convex aspect of the adrenal cortex and then expressing the adrenal medulla through the incision by mild pressure on the concave aspect of the adrenal. The adequacy of adrenal demedullation was verified by the measurement of plasma E following decapitation, a potent stimulus to adrenal E release. The rats were allowed to recover for 1 week, then each of nine bilaterally demedullated rats received one dose of 6-hydroxydopamine (6-OHDA; Sigma, St. Louis, MO) 20 mg/kg i.p. followed 24 hr later by four doses on 4 successive days of reserpine (Sigma) 5 mg/kg i.p. dissolved in 10% ascorbic acid in 0.9% saline. This treatment was designed to lower circulating E by demedullation, destroy sympathetic nerve terminals with 6-OHDA and eliminate catecholamine storage in any nerve terminals which survived 6-OHDA with reserpine treatment. Rats treated with adrenal demedullation, 6-OHDA and reserpine are referred to as D-6-R rats. All rats were sacrificed by decapitation 24 hr after the last dose of reserpine was given and a blood sample was collected from each rat into a heparinized tube. Erythrocytes were separated and the plasma was stored at -70°C. The iris was separated, washed with ice-cold saline and stored at -70°C. Adrenal glands, brainstems and livers were separated.

From the Department of Medicine, Division of Nephrology, University of California San Diego Medical Center.

* Dr. Elayan is a visiting Associate Professor of Pharmacology from the University of Jordan.

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Reprint requests: Michael G. Ziegler, MD, UCSD Medical Center, 225 Dickinson Street, H-781-B, San Diego, CA 92103.
from the sham-operated rats, washed with ice-cold saline and stored at —70°C. Tissues were homogenized in equal volume of 0.1 M Tris HCl buffer with 0.1% triton pH 7, then diluted to form 100 mg per ml, centrifuged for 10 min at 6000 g and the supernatant stored at —70°C.

Catecholamines were assayed in duplicate 100 μl samples of tissue homogenates or plasma according to the COMT radioenzymatic assay of Ziegler et al. N-methylating enzymes activities were assayed in 50 μl duplicates of tissue homogenized according to the radioenzymatic method of Ziegler et al using norepinephrine (NE) 10^{-3} M as substrate in the presence or absence of SKF 29661 (10^{-4} M) (Smith Kline and French, Philadelphia, PA). Dopamine (DA) and NE, both at the concentration of 10^{-4} M, were used as substrates in certain determinations and the ratios of epinephrine formed from DA to E formed from NE were determined.

The results were expressed as mean values, standard error of the mean (SEM). The student t-test, one-way analysis of variance and Duncan’s test were used to evaluate the level of statistical significance.

**Results**

A 10^{-4} M concentration of the PNMT inhibitor, SKF 29661, inhibited E formation in adrenal gland and brainstem (P < 0.001) but not in the liver (Fig. 1). SKF 29661 inhibited iris E-forming activity slightly less than adrenal enzyme activity. DA (10^{-4} M) was used as a substrate and the amount of [3-H] epinephrine formed from [3-H] S-adenosylmethionine in the presence of iris homogenate was compared with the amount of [3-H] E formed from NE (10^{-4} M) in an aliquot of the same tissue. The ratio of the product formed from DA compared to the product formed from NE (DA:NE ratio) served as a guide to the specificity of an enzyme for phenylethanolamines such as NE. The DA:NE ratio for the normal iris was similar to that of adrenal and brainstem (Table 1), but increased from 0.06 to 3.3 when iris was incubated with the PNMT inhibitor SKF 29661.

Unilateral superior cervical ganglionectomy decreased NE content of the iris by 63% compared to the contralateral side (P < 0.05). Even larger reductions occurred in denervated muscle and salivary gland where NE was at 12% and 9%, respectively, of the levels found in the contralateral side. In contrast, E, DA and E forming activity were not diminished (Fig. 2).

Bilateral adrenal demedullation did not affect E-generating activity (Fig. 3) or catecholamine content (Fig. 4) in the iris. Treatment with D-6-R did not significantly alter E-generating activity (Fig. 3) or E levels in the iris even though NE levels decreased dramatically in response to D-6-R (Fig. 4).

The rats were sacrificed by decapitation, a powerful stimulus to catecholamine release, as seen in Figure

**Table 1. N-methylating enzyme activities**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>NE 10^{-4} M</th>
<th>DA 10^{-4} M</th>
<th>DA/NE ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal</td>
<td>3.7 ± 0.35 × 10^4</td>
<td>449 ± 68</td>
<td>0.012 ± 0.01</td>
</tr>
<tr>
<td>Brainstem</td>
<td>34.6 ± 12.8</td>
<td>0.18 ± 0.03</td>
<td>0.005 ± 0.004</td>
</tr>
<tr>
<td>Iris</td>
<td>63.24 ± 0.44</td>
<td>3.61 ± 1.0</td>
<td>0.06 ± 0.24</td>
</tr>
<tr>
<td>Liver</td>
<td>3.81 ± 0.44</td>
<td>10.87 ± 0.97</td>
<td>2.8 ± 0.12</td>
</tr>
</tbody>
</table>

The number of pmol/g/hr of NE converted to E or of DA converted to epinephrine when 10^{-4} M substrate was incubated with tissue homogenates. The last column gives the ratio of the rate of conversion of the two catecholamines.
5. Bilateral adrenal demedullation decreased plasma NE ($P < 0.01$) and E ($P < 0.001$) but increased plasma DA levels ($P < 0.05$). The addition of 6-OHDA and reserpine to adrenal medullectomy further lowered plasma NE levels ($P < 0.05$).

**Discussion**

The iris formed E at about twice the rate of brainstem homogenate (Table 1). Enzyme activity in the iris was fairly selective for the phenylethanolamine NE when compared with the phenylethylamine DA. While brainstem methylated DA only 1% as well as NE, iris methylated DA at about 6% of the rate at which it methylated NE. This specificity for NE over DA is a characteristic of the enzyme PNMT. When a PNMT inhibitor was added to iris homogenate, it lost all specificity for NE and actually methylated DA better than NE. These results suggest that the E-forming activity of iris is predominantly PNMT and the remainder is in a nonspecific N-methyltransferase that methylates both NE and DA. The addition of $10^{-4}$ M SKF 29661 to iris homogenate caused an inhibition of E-forming activity parallel to, but slightly less than that of adrenal (Fig. 1). This is also compatible with a small amount of NMT unaffected by SKF 29661 and a larger amount of PNMT.

The adrenal medulla forms E from PNMT in spe-
cialized postganglionic sympathetic neurons and some sympathetic ganglia contain PNMT. However, the PNMT in iris appears to be extraneuronal. Superior cervical ganglionectomy reduced NE content of the iris to 37% of the contralateral side while E-forming activity, E and DA contents were not affected (Fig. 2). Removal of the adrenal medullae failed to lower iris E while D-6-R diminished iris E by about half and iris NE by 98%. Extraneuronal PNMT has also been reported in rat retina, rat brain and human psoriatic skin. This extraneuronal PNMT does not appear to require intraneuronal NE to serve as substrate to form E. Most tissue NE is stored in nerve vesicles used to store NE, since denervation had no effect on NE content of facial skeletal muscles and salivary gland (Fig. 3).

Treatment with D-6-R markedly depleted iris NE, but unilateral superior cervical ganglionectomy depleted iris NE by 63%. Rat retina also responds to unilateral superior cervical ganglionectomy with only partial NE depletion, possibly due to bilateral innervation of the retina by the superior cervical ganglion. This explains why NE content of iris and eyelid was less reduced than NE content of facial skeletal muscles and salivary gland (Fig. 3).

Our results suggest that at least half of the E in the iris is synthesized extraneuronally in the iris. Removal of both adrenal medullae dramatically diminished plasma E, yet had no effect on iris E. During surgical and chemical denervation the capacity of iris to synthesize E remained intact. Much of the E present in the iris appears to be stored outside of nerve vesicles used to store NE, since denervation had no effect on E levels. NE is extensively cleared from the circulation by uptake-1 processes into sympathetic nerves while E is primarily cleared by uptake-2 processes into smooth muscle and the vasculature, which are likely storage sites for E made in either the adrenal or iris. These catecholamine uptake processes are so rapid that plasma catecholamines have a circulating half-life of only a few minutes. Tissue E is metabolized by the enzyme catechol-O-methyltransferase and most E eventually appears in the urine as an O-methylated metabolite. This implies that E is continually synthesized to maintain tissue stores of E. Although D-6-R treatment markedly depleted iris NE, most neuronal NE is stored in nerve vesicles inaccessible to PNMT. The amount of free NE available to metabolizing enzymes such as PNMT should have decreased less than NE stored in nerve vesicles since reserpine depletes vesicles of stored NE. The disruption of sympathetic nerve terminals by D-6-R apparently left sufficient free tissue NE to serve as a substrate for PNMT to synthesize E.

Epinephrine is much better than NE at stimulating β2 receptors, and β2 adrenergic receptors exert the predominant adrenergic influence on aqueous humor dynamics. It alters uveoscleral flow, aqueous humor formation and intraocular pressure. β adrenergic receptors mediate inhibition of iris sphincter tone. Intraocular 6-OHDA treatment has been used in man and we find that 6-OHDA depletes NE, but not E or PNMT in the iris. Synthesis of E by the iris may alter the course of iritis and glaucoma.

Key words: epinephrine, iris, norepinephrine, phenylethanolamine-N-methyltransferase, PNMT

References