Prejunctional Modulation of Norepinephrine Release in the Human Iris–Ciliary Body

James E. Jumblatt,* S. Edet Ohia,† and Rita C. Hackmiller*

**Purpose.** To characterize the prejunctional mechanisms that control the impulse-evoked release of norepinephrine in the isolated, superfused human iris–ciliary body.

**Methods.** Human iris–ciliary body tissue segments were preincubated with 3H-norepinephrine, superfused and electrically-stimulated in vitro to evoke the discharge of 3H-norepinephrine. The effects of prejunctional modulators on evoked 3H-norepinephrine overflow were evaluated.

**Results.** Stimulation-evoked (but not spontaneous) 3H-norepinephrine release was inhibited by α2-adrenergic, muscarinic, dopaminergic, neuropeptide Y, and prostaglandin agonists and was enhanced by angiotensin II. Agonist-induced effects on 3H-norepinephrine overflow were blocked by selective antagonists, where available. Yohimbine and atropine alone enhanced 3H-norepinephrine output, suggesting that prejunctional α2-adrenergic and muscarinic receptors undergo tonic activation by endogenously released neurotransmitters.

**Conclusions.** Human ocular sympathetic nerves express inhibitory α2-adrenergic, muscarinic, dopaminergic, prostaglandin, and neuropeptide Y receptors and facilitatory angiotensin II receptors that control the impulse-evoked release of 3H-norepinephrine. These receptors may be useful targets for pharmacologic manipulation of the adrenergic system in vivo. Invest Ophthalmol Vis Sci 1993;34:2790–2793.

**The release of norepinephrine (NE) from peripheral sympathetic nerves is subject to local regulation by endogenous neurotransmitters, autacoids and hormones acting at prejunctional receptors.** Previous studies have demonstrated that sympathetic terminals in the rabbit iris–ciliary body contain prejunctional muscarinic, α2-adrenergic, neuropeptide Y, prostaglandin, and angiotensin II receptors that modulate NE secretion in vitro. Little information exists, however, on the mechanisms that control sympathetic neurotransmitter release in the human eye. Recently, we developed in vitro methods to measure electrically-evoked secretion of 3H-norepinephrine (3H-NE) from isolated, superfused segments of human iris–ciliary body and have used this system to evaluate the modulatory effects of prostaglandins on evoked 3H-NE release.

Presented here is evidence that human ocular sympathetic nerves express, in addition to prostaglandin receptors, inhibitory α2-adrenergic, muscarinic, dopaminergic, and neuropeptide Y receptors and facilitatory angiotensin II receptors that are functionally analogous to those described in other systems.

**METHODS**

Field-stimulated secretion of 3H-NE from isolated, superfused human iris–ciliary body segments was measured as previously described, with minor modifica-
Iris-ciliary bodies were dissected from enucleated human eyes (donor ages 10–86 years; mean age 59 ± 3 years; n = 43) obtained within 3–6 hours of death from the Kentucky Lions Eye Bank and were incubated for 60 minutes at 37°C in Krebs-Ringer buffer (composition in mM: NaCl, 118; KCl, 4.8; CaCl₂, 1.3; KH₂PO₄, 1.2; NaHCO₃, 25; MgSO₄, 2.0; dextrose, 10; indomethacin, 0.003; Na ascorbate, 0.1; pH 7.3) containing 2.5 μCi/ml l-[7,8-³H]norepinephrine (40–50 μCi/mmol; Amersham, Arlington Hts., IL). The incubation medium was infused continuously with 95% O₂/5% CO₂ gases. After labeling, tissues were rinsed for 15 minutes in three changes of 50 ml buffer to remove excess radioactivity and then cut equatorially into halves. Individual tissue segments were transferred to plastic superfusion chambers and superfused at a rate of 2 ml/min with oxygenated buffer containing 1 mM desipramine to inhibit neuronal reuptake of catecholamines. After 30 minutes of superfusion to reduce spontaneous ³H efflux to acceptable levels, fractions of the superfusate were collected at 5-minute intervals. Aliquot portions (4 ml) of each fraction were combined with 14 ml scintillation cocktail (Ecolume; ICN Biochemicals, Irvine, CA) and analyzed for radioactivity by liquid scintillation spectrometry.

Release of ³H-NE was elicited by consecutive trains of electrical field-stimulation, each train consisting of 300 rectangular pulses (5 Hz, 1 msec pulse duration, 50 V/cm inter-electrode distance) delivered to the platinum chamber electrodes with a Grass S48 pulse stimulator (Grass Instruments, Quincy, MA).

FIGURE 1. Electrically-evoked discharge of ³H-NE in isolated, superfused segments of human iris—ciliary body. Stimulation (S₁-S₂)-induced ³H efflux is indicated by the shaded peak areas above the extrapolated basal ³H efflux. Tissues were stimulated twice at 20 minutes (S₁) and 55 minutes (S₂) after beginning fraction collection. Net, stimulus-evoked ³H-NE output was calculated by subtraction of extrapolated basal (spontaneous) ³H efflux from total ³H overflow in the five fractions after each stimulation train (see Fig. 1, shaded peak areas). Basal ³H efflux was assumed to decline linearly between the fraction immediately preceding stimulation and the fifth fraction after stimulation. Stimulation-evoked ³H-NE outflow was found previously to be calcium-dependent and sensitive to antagonists of voltage-activated calcium and sodium channels. Chromatographic analysis of the radioactive release products indicated that most (>80%) of the ³H-labeled material secreted in response to field-stimulation consists of unmetabolized ³H-NE, whereas the spontaneously released ³H represents various NE metabolites and oxidation products. Field-stimulated ³H efflux was thus designated as evoked ³H-NE release.

Test drugs were added 10 minutes before and during S₂. Peak ratios of stimulation-evoked ³H-NE overflow (S₂/S₁) were calculated and compared to the means of the corresponding values from separate control preparations. In general, experimental and control studies were performed in parallel using tissue segments obtained from the same donor, although not always from the same eyes. Results are expressed as the percentage of change in the peak ratios of evoked ³H-NE release relative to the corresponding control values; means ± SE. Statistical significance of drug-mediated effects on ³H-NE release was evaluated by Student’s t test (two-tailed, unpaired data). Differences with P values <0.05 were accepted as significant.

The prostaglandins PGE₂ and PGF₂α were purchased from Cayman Chemical Co. (Ann Arbor, MI). Bromocryptine was purchased from Research Biochemicals Inc. (Natick, MA). All other drugs were purchased from Sigma Chemical Co. (St. Louis, MO).

RESULTS

As illustrated by the example in Figure 1, electrical field-stimulation of human iris—ciliary body segments preincubated with ³H-NE elicited peaks of increased ³H-NE output above a declining baseline of spontaneous ³H efflux. The average peak ratio of stimulus-evoked ³H-NE overflow in untreated controls was S₂/S₁ = 0.97 ± 0.04 (n = 16).

A series of representative α₂-adrenergic, dopaminergic, muscarinic, neuropeptide Y, prostaglandin, and angiotensin II agonists and antagonists were examined for their modulatory effects on stimulus-evoked ³H-NE release. As shown in Figure 2, evoked ³H-NE release was inhibited 29% by the α₂-adrenergic agonist clonidine (10⁻⁶ M), 25% by the dopamine agonist bromocryptine (10⁻⁶ M), and 27% by neuropeptide Y.
edly enhanced evoked 3H-NE release, suggesting that yohimbine modify the response to bromocryptine (not selective α2-adrenoceptor antagonist yohimbine and the field stimulation. Haloperidol (10⁻⁷ M) alone did not significantly modify 3H-NE release.

prejunctional α2-adrenoceptors receptors undergo stimulation by endogenous NE released during tonic stimulation. Haloperidol (10⁻⁷ M) alone did not significantly modify 3H-NE release.

As shown in Figure 3, evoked 3H-NE release was reduced 56% by exogenous muscarine (5 × 10⁻⁶ M), the effects of which were antagonized by atropine (10⁻⁷ M). Atropine (10⁻⁶ M) alone significantly enhanced evoked 3H-NE release, suggesting that prejunctional α2-adrenoceptors receptors undergo tonic stimulation by endogenous NE released during field stimulation. Haloperidol (10⁻⁷ M) alone did not significantly modify 3H-NE release.

As shown in Figure 3, evoked 3H-NE release was reduced 56% by exogenous muscarine (5 × 10⁻⁶ M), the effects of which were antagonized by atropine (10⁻⁷ M). Atropine (10⁻⁶ M) alone significantly enhanced evoked 3H-NE release by 33%, suggesting that tonic cholinergic inhibition of 3H-NE release occurs in this system. In accordance with previous results, exogenous PGE₂ (10⁻⁶ M) strongly inhibited evoked 3H-NE release, whereas PGF₂α (10⁻⁶ M) had no effect. The octapeptide angiotensin II (10⁻⁹ M) enhanced evoked 3H-NE release by 35%. The response to angiotensin II was abolished by the selective angiotensin II antagonist saralasin (10⁻⁷ M), which alone produced no effect. None of the above agents significantly modified spontaneous 3H output (not shown).

**DISCUSSION**

During the past two decades, more than a dozen different types of prejunctional receptors have been found to modulate NE secretion in various autonomic end organs. Some of these receptors (e.g., prejunctional α2-adrenoceptors mediating autofeedback control of NE release) appear to be present on virtually all sympathetic nerve endings, whereas others are variably distributed depending on the target organ and/or species. The current results provide the first evidence that human ocular sympathetic nerves express modulatory α2-adrenergic, muscarinic, dopaminergic, neuropeptide Y, and angiotensin II receptors, in addition to inhibitory prostaglandin receptors described in a previous study. Although their physiologic roles remain to be defined, these receptors appear to be functionally and pharmacologically analogous to those described in the rabbit iris-ciliary body, cat nictitans and various human nonocular tissues. The results of this study offer new perspectives for pharmacologic manipulation of sympathetic neurotransmission in the human eye.

Under the assay conditions employed in this study, prejunctional α2-adrenoceptors appeared to undergo considerable tonic activation by endogenously secreted NE, as indicated by the facilitative effect of yohimbine on evoked 3H-NE release. Previous work suggests that autoinhibition of NE release mediated by prejunctional α2-adrenoceptors is favored by high stimulation frequencies (>5 Hz) and the presence of catecholamine reuptake inhibitors, both of which tend to elevate the biophase concentration of released NE. Moreover, an inverse relationship has been observed between the level of tonic α2-adrenoceptor stimulation and the prejunctional effects of exogenous α2-agonists, which are superimposed on those of endogenously released NE. Other studies indicate that simultaneous activation of prejunctional α2-adrenoceptors may attenuate the responses to other inhibitory receptors located on the same termi...
nals, suggesting that different receptors may share a common pathway for regulation of NE secretion.

The observed facilitation of evoked $^3$H-NE release by atropine suggests that prejunctional muscarinic receptors undergo activation by endogenous acetylcholine released in response to field stimulation. In support of this interpretation, field-stimulated release and overflow of acetylcholine has been demonstrated in rabbit and guinea pig irides pretreated with acetylcholinesterase inhibitors. In the absence of such inhibitors (e.g., in this study), one might expect that the modulatory influence of endogenously secreted acetylcholine on NE release would be limited to nearby adrenergic terminals. Consistent with this idea, the evidence for prejunctional cholinergic regulation of sympathetic neurotransmission comes mainly from studies of cardiovascular preparations (e.g., blood vessels and the sinoatrial region of the heart) in which postganglionic sympathetic and parasympathetic fibers lie in close proximity. A similar close apposition between adrenergic and cholinergic fibers has been described in the iris dilator muscle, which receives substantial inhibitory cholinergic innervation. However, it is unclear what portion of field-stimulated $^3$H-NE output originates in this structure versus other regions of the iris–ciliary body (e.g., the ciliary processes), and a meaningful evaluation of the physiologic significance of the observed cholinergic inhibition of $^3$H-NE release will require more precise information on the sites of origin of the released acetylcholine and the sympathetic terminals with which it interacts.

Prejunctional receptors have been postulated to mediate the intraocular pressure-lowering effects of several drugs, including $\alpha_2$-adrenergic and dopaminergic agonists. Direct investigation of prejunctional receptor involvement in ocular drug responses is hampered by the presence of postjunctional receptors of similar type in ocular tissues. However, recent evidence suggests that some prejunctional receptors (e.g., prostanoid EP$_3$ receptors) may differ in subtype from their postjunctional counterparts. Such differences might allow the use of subtype-selective agents to probe the physiologic and pharmacologic roles of prejunctional receptors in vivo.

Key Words
Prejunctional receptors, norepinephrine, neurosecretion, sympathetic nerves, human iris–ciliary body

References