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 $^3$H-norepinephrine, superfused and electrically-stimulated in vitro to evoke the discharge of $^3$H-norepinephrine. The effects of prejunctional modulators on evoked $^3$H-norepinephrine overflow were evaluated.

Results. Stimulation-evoked (but not spontaneous) $^3$H-norepinephrine release was inhibited by $\alpha_2$-adrenergic, muscarinic, dopaminergic, neuropeptide Y, and prostaglandin agonists and was enhanced by angiotensin II. Agonist-induced effects on $^3$H-norepinephrine overflow were blocked by selective antagonists, where available. Yohimbine and atropine alone enhanced $^3$H-norepinephrine output, suggesting that prejunctional $\alpha_2$-adrenergic and muscarinic receptors undergo tonic activation by endogenously released neurotransmitters.

Conclusions. Human ocular sympathetic nerves express inhibitory $\alpha_2$-adrenergic, muscarinic, dopaminergic, prostaglandin, and neuropeptide Y receptors and facilitatory angiotensin II receptors that control the impulse-evoked release of $^3$H-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.1 Previous studies have demonstrated that sympathetic terminals in the rabbit iris–ciliary body contain prejunctional muscarinic, $\alpha_2$-adrenergic, neuropeptide Y, prostaglandin, and angiotensin II receptors that modulate NE secretion in vitro.2–6 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 $^3$H-norepinephrine ($^3$H-NE) from isolated, superfused segments of human iris–ciliary body and have used this system to evaluate the modulatory effects of prostaglandins on evoked $^3$H-NE release.7 Presented here is evidence that human ocular sympathetic nerves express, in addition to prostaglandin receptors, inhibitory $\alpha_2$-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 $^3$H-NE from isolated, superfused human iris–ciliary body segments was measured as previously described,7 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 μM 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).

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 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).
edly enhanced evoked[^3H]-NE release, suggesting that yohimbine modify the response to bromocryptine (not shown). Haloperidol (10^{-7} M) alone did not significantly modify[^3H]-NE release.

Prejunctional a2-adrenoceptors receptors undergo nonselective dopaminergic antagonist haloperidol shown). Addition of yohimbine (10^{-7} M) alone markt did not antagonize the response to clonidine, nor did bromocryptine were blocked, respectively, by the selective angiotensin II antagonist saralasin (10^{-7} M). The prejunctional effects of clonidine and haloperidol (both at 10^{-7} M). At these concentrations, haloperidol did not antagonize the response to clonidine, nor did yohimbine modify the response to bromocryptine (not shown). Addition of yohimbine (10^{-7} M) alone markedly enhanced evoked[^3H]-NE release, suggesting that prejunctional a2-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,7 exogenous PGE_{2} (10^{-6} M) strongly inhibited evoked[^3H]-NE release, whereas PGF_{2a} (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).

FIGURE 2. Effects of a2-adrenergic, dopaminergic and neuropeptide Y compounds on stimulation-evoked[^3H]-NE release. Abbreviations and drug concentrations: CLON = clonidine, 10^{-6} M; YOH = yohimbine, 10^{-7} M; BROM = bromocryptine, 10^{-6} M, HAL = haloperidol, 10^{-7} M, NPY = neuropeptide Y, 10^{-7} M. Vertical bars and error bars represent the means ± SEM; numbers of determinations shown in parentheses. *P < 0.05 compared with untreated controls.

**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.1 Some of these receptors (e.g., prejunctional a2-adrenoceptors mediating autofeedback contr-
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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