The effects of neuropeptide Y (NPY) were studied on an in vitro preparation of rabbit iris dilator muscle. NPY by itself (10^{-11} M to 10^{-6} M) had no effect on the resting tension or on the maximal electrically-induced response (MER) of the dilator. Phenylephrine (10^{-9} M to 10^{-4} M) caused a dose-dependent contraction of the dilator muscle (7.8% to 40.6% of the MER). The addition of NPY 10^{-6} M enhanced the phenylephrine-induced muscle contraction (8.8% to 76.8% of the MER) without altering the E_Ca value (5 \times 10^{-4} M) of the phenylephrine dose-response curve. These findings support a modulatory role for NPY on the iris dilator muscle. Invest Ophthalmol Vis Sci 29:330–332, 1988

Many biologically active peptides have been localized to nerve fibers supplying the intraocular muscles. Neuropeptide Y (NPY), a 36-amino acid peptide, is co-stored with norepinephrine in terminal vesicles of sympathetic neurons. The mammalian iris dilator muscle is richly innervated by nerve fibers containing NPY, evidently derived from the ipsilateral superior cervical ganglion. NPY has varied functional effects including adrenergic interactions in many tissues, but its role in the eye is not well understood. We now report the interaction of NPY with adrenergic mechanisms in the rabbit iris dilator muscle in vitro.

**Materials and Methods.** Albino rabbits weighing 2 to 3 kg were sacrificed by air embolism. The enucleated globe was bisected at the equator and the lens removed using a dissecting microscope. Wedge sections of the iris including as much of the root as possible were suspended horizontally in a specially designed 0.5 ml plexiglass chamber. The iris root was fixed with compression pins to one end of the chamber, and the sphincter muscle of the tissue was hooked with a microsurgical needle (Alcon CU-5; Fort Worth, TX) attached to a 10-0 nylon suture. The suture was tied to a Grass FT03 force-displacement transducer (Grass Instruments, Quincy, MA) mounted on a micromanipulator. The bathing solution was Heps buffer (mM composition: NaCl 117.9, NaHCO_3 11.8, KH_2PO_4 1.2, Heps 7.5, HEPES Na 7.9, CaCl_2 2.5, MgSO_4 1.2, KCl 4.7, dextrose 181.6) at room temperature with continuous bubbling with a 95% O_2/5% CO_2 gas mixture (pH 7.3–7.4). Isometric muscle tensions were recorded on a Grass Polygraph (Model RPS 11A). The iris tissue was mounted so that the dilator muscle fibers were oriented parallel to the axis of the force-displacement transducer; the attached sphincter muscle was oriented perpendicularly to eliminate any effect of sphincter muscle contractions on the transducer displacement. The addition of pilocarpine 10^{-5} M to the preparation did not alter the radial dilator muscle tension (unpublished observation). A Grass field stimulator (Model S6C) was used to induce electrical muscle contractions for each preparation (electrical parameters: 20 V, 2 msec, 20 Hz) through platinum electrodes spaced 4 mm apart and placed above and below the mounted tissue. In preliminary experiments, variation of the parameters of electrical stimulation defined conditions (20 V, 2 msec, 20 Hz) that resulted in maximum muscle contraction in all preparations. These field stimulation parameters were then applied to each preparation to determine the maximum muscle contraction in response to electrical stimulation (MER). The MER was used as a physiologic response, against which pharmacologic effects were compared. Analysis of drug effects on submaximal levels of electrical stimulation were not studied specifically.

Baseline tissue tension was adjusted using the micromanipulator to an average (mean ± SEM) of 34.3 ± 2.8 mg for the control tissues and 38.2 ± 3.0 mg for the experimental tissues (P > 0.5, student t-test). After measuring the MER for each preparation, the bathing solution was removed and replaced with a 0.5 ml aliquot of Heps buffer containing NPY, 1-phenylephrine or a combination of the two. Drugs were studied in increasing concentrations; tissues were exposed to the agents for at least 5 min at each concentration. Pharmacologically-induced contractions were expressed as a percentage of the maximum electrical response (MER) for each tissue preparation. Values are expressed as the mean ± SEM. Student t-test for unpaired data was used for statistical analysis. Our experiments conformed to the ARVO Resolution on the Use of Animals in Research.

**Results.** Repeat exchange of control bathing solution had no effect on baseline muscle tension. The addition of NPY by itself (10^{-11} M to 10^{-6} M) did not alter either the resting dilator muscle tension or the MER.

Phenylephrine (10^{-9} M to 10^{-4} M) caused a dose-dependent increase in contraction of the iris dilator muscle which ranged from 7.8 ± 2.6% to 40.6 ± 11.2% of MER (Fig. 1). The addition of NPY 10^{-6}
M to the phenylephrine solutions (10^{-9} M to 10^{-4} M) resulted in a potentiation of the phenylephrine response: 8.8 ± 4.2% to 76.9 ± 17.5% of MER (Fig. 1). The NPY potentiation was statistically significant (P < 0.05) at phenylephrine concentrations of 10^{-6} M and greater.

The effect of increasing concentrations of NPY (10^{-11} M to 10^{-6} M) was studied on muscle contraction induced by a 10^{-5} M phenylephrine. Phenylephrine contraction was not significantly (P > 0.4) altered by the presence of NPY 10^{-11} M. However, the addition of NPY at a concentration of 10^{-10} M and greater resulted in enhanced muscle contraction (Table 1). The simultaneous addition of clonidine (10^{-5} M), an α2-adrenergic agonist, did not alter the NPY (10^{-7} M) enhancement of phenylephrine-induced (10^{-5} M) contraction of the dilator muscle. Clonidine alone had no effect on the resting dilator muscle tension or the MER.

**Discussion.** Interactions between NPY and the adrenergic system are now well known, varying with the tissue and species under study. For instance, NPY inhibits contractions of the rodent vas deferens by acting presynaptically on adrenergic nerves to depress the release of norepinephrine.6-8 This presynaptic effect appears to be mediated by an NPY-specific receptor.9 On vascular smooth muscles of the cat submandibular gland, however, NPY has a direct contractile effect. NPY-induced vasoconstriction here is slower in onset, longer in duration and less efficacious than the response to norepinephrine and is resistant to α-adrenergic blockade.10 On isolated rabbit, guinea pig, or rat femoral, gastric and mesenteric arteries, NPY is not a potent contractile agent; but it enhances the electrically- and adrenergically-induced contractions of these vessels.11,12 In contrast to the rat vas deferens, NPY appears to act on peripheral vascular smooth muscle by a nonadrenergic postsynaptic mechanism of action, possibly also by an NPY-specific receptor.13

Unlike its activity on some vascular smooth muscles, NPY alone has no direct effect on the isolated rabbit iris dilator muscle. In addition, it does not alter maximal electrically-induced contractions of the dilator. Yet, this peptide augments the dose-dependent contractile effect of the α-adrenergic agonist phenylephrine while not affecting the EC50 value (effective concentration resulting in a 50% response). These observations are consistent with postsynaptic modulation of the dilator muscle by NPY.

The observed NPY effect on the dilator is not attributable to presynaptic reduction of norepinephrine release since NPY enhances the functional response to phenylephrine. We used clonidine, a moderately selective α2 agonist, to test further whether NPY might function as a competitive α2-adrenergic antagonist. Since clonidine 10^{-5} M alone does not effect the iris dilator muscle, this agent does not act as an α1 agonist in our assay system. If the NPY-enhanced phenylephrine-induced contraction was functioning as an α2-adrenergic receptor antagonist, we would

**Table 1. The effect of NPY on phenylephrine-induced contraction of the isolated rabbit dilator muscle**

<table>
<thead>
<tr>
<th>NPY concentration (M)</th>
<th>10^{-11}</th>
<th>10^{-10}</th>
<th>10^{-9}</th>
<th>10^{-8}</th>
<th>10^{-7}</th>
<th>10^{-6}</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Enhancement of muscle contraction (±SEM)</td>
<td>11.4 ± 23%</td>
<td>21.8 ± 7%†</td>
<td>33.6 ± 7%†</td>
<td>33.1 ± 10%†</td>
<td>121.0 ± 20%†</td>
<td>70.0 ± 22%†</td>
</tr>
</tbody>
</table>

* Muscle contraction is measured with 10^{-5} M phenylephrine concentration and an increasing concentration of NPY (10^{-11} to 10^{-4} M). N = 5 for each concentration.
† Significant versus phenylephrine control, t-test, P < 0.01.

% Enhancement of muscle contraction = % MER with NPY and phenylephrine
% MER with phenylephrine = 100 × (% MER with phenylephrine - 1)
expect that the competition for $\alpha_2$ receptor occupancy by the simultaneous addition of clonidine would eliminate this effect and demonstrate a final contraction of the dilator that was equal to or less than that obtained by phenylephrine alone. In fact, the addition of clonidine did not alter the NPY-enhanced phenylephrine-induced contraction of the dilator, thus making it unlikely that NPY functions as an $\alpha_2$-adrenergic receptor antagonist in our system.

Further experiments clearly are needed to define the mechanism by which NPY acts on the iris dilator muscle. A post-junction receptor, perhaps NPY-specific, may well mediate its effects. While NPY does not seem to act on the $\alpha_2$-adrenergic receptor, our data do not exclude the possibility of a different presynaptic receptor mechanism to facilitate norepinephrine release.

Key words: neuropeptide Y, iris dilator, muscle contraction, phenylephrine, adrenergic nervous system

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References


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Synthesis of Prostaglandin E in Rabbit Eyes With Topically Applied Epinephrine

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Using radioimmunoassay techniques, we measured the amounts of prostaglandin E (PGE) in the aqueous humor and vitreous body of 22 phakic and ten aphakic rabbit eyes. Either epinephrine and placebo, epinephrine and indomethacin, indomethacin and placebo, or placebo and placebo were administered topically for 5 months. Treatment of aphakic eyes was initiated 1 month after intraocular lens extraction. Topically applied epinephrine apparently in-duced the synthesis of prostaglandin, manifested by elevated PGE in the aqueous and vitreous. Phakic eyes treated with epinephrine and placebo had mean PGE levels of 407.33 pg/ml in the aqueous and 177.0 pg/ml in the vitreous, whereas control eyes given only placebo had mean levels of 165.83 in aqueous and 59.17 in vitreous. Topically applied indomethacin inhibited epinephrine-induced synthesis of PGE in the aqueous humor, but had no significant