Nitric Oxide*

Role as a Relaxant Agonist and Transmitter of Nonadrenergic Noncholinergic Inhibitory Nerves in Guinea Pig Trachea

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Nitric oxide (NO) has been proposed as an inhibitory neurotransmitter in guinea pig trachea. Studies were undertaken to examine the relaxant actions of NO and its possible role as a transmitter of nonadrenergic noncholinergic inhibitory (NANC) nerves and as a modulator of contractile responses in guinea pig trachea. Isometric responses were measured in paired segments of trachea and aorta mounted in organ baths filled with Krebs-Henseleit solution containing indomethacin, maintained at 37°C and gassed with 5% CO₂ in O₂. All relaxant responses were determined in precontracted tissues. The NANC responses were evoked by electrical field stimulation (EFS) in tracheal segments treated with guanethidine, propranolol, and atropine. Effects of endogenously released NO were determined by treatment of tracheal segments with the NO synthase inhibitor, Nω-nitro-L-arginine (NNA), prior to administration of contractile agonists.

Concentration-dependent relaxations were produced in all tissues with trachea being approximately 100-fold less sensitive than aorta. Effects of agents which potentially modify NO half-life in the smooth muscle biophase were examined on NO and NANC responses. Relaxant effects of NO in aorta were enhanced by superoxide dismutase (SOD) or L-cysteine and attenuated by pyrogallol, ascorbate, or hydroquinone. The SOD prevented the inhibitory effect of pyrogallol. In trachea, relaxant effects of NO were attenuated by hydroquinone or ascorbate but unaffected by SOD, pyrogallol, or L-cysteine. The NANC responses were enhanced by ascorbate, inhibited by hydroquinone or pyrogallol, and unaffected by L-cysteine or SOD. Treatment of tracheal segments with NNA to inhibit NO synthesis failed to alter contractile responses to acetylcholine, histamine, leukotriene D₄, or substance P in control trachea or to antigen challenge in sensitized trachea.

Results of the present study indicate the following: (1) NO-induced relaxations in the trachea are modulated differently from those in the aorta; (2) endogenous superoxide generation does not account for the relative insensitivity of trachea to the relaxant effects of NO; (3) NANC and NO-induced responses in the trachea do not mimic one another in relation to the treatments applied; and (4) endogenous NO does not appear to influence the ability of guinea pig tracheal smooth muscle to contract nor does it modify antigen-induced mediator release.

Catalytic Autoantibodies to Vasoactive Intestinal Peptide*

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We have previously described an autoantibody-catalyzed hydrolysis of vasoactive intestinal peptide (VIP), a 28-amino acid neuropeptide with airway smooth muscle relaxant and anti-inflammatory properties.¹ Catalytic antibodies can be expected, by definition, to achieve more efficient inactivation of VIP than antibodies that bind the peptide stoichiometrically. Our aims here were the characterization of catalytic autoantibodies to VIP in asthma; and molecular cloning of catalytic anti-VIP antibody.

METHODS

Cleavage of VIP labeled at tyrosine¹⁰ with¹²⁵I was determined by high performance liquid chromatography or acid precipitation. Antibodies were purified from plasma by affinity chromatography on immobilized protein G and VIP. The cDNA for a murine anti-VIP single chain Fv (scFv) and its L-chain subunit² was cloned in a phagemid vector and the recombinant proteins were purified by metal-affinity chromatography.

RESULTS AND DISCUSSION

The IgG from >50% asthma patients (N 35) displayed significant hydrolysis of VIP, compared to <10% nonasthmatic control subjects (N 19). Four IgG samples each from asthmatics and nonasthmatics were analyzed for reaction kinetics. In each case, Michaelis-Menten kinetics was observed. The rates of peptide hydrolysis by antibodies from asthmatics were consistently greater than those from nonasthmatics. The kinetic efficiency of VIP-specific antibodies from an asthmatic (kcatalyst/km 9.6×10⁷ M⁻¹sec⁻¹) was within an order of magnitude of that for chymotrypsin. Taken together with previous observations,⁵ it appears that two types of antibodies are associated with asthma: a high affinity, noncatalytic antibody, and a low affinity, catalytically-efficient antibody. Both types of an-

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