Vasoactive Intestinal Peptide: A Possible Transmitter of Nonadrenergic Relaxation of Guinea Pig Airways

Abstract. *Vasoactive intestinal peptide, a smooth-muscle relaxant neuropeptide with neurotransmitter properties, was released during electrical field stimulation of guinea pig trachea. The amount released correlated with the degree of relaxation, and the release was blocked by tetrodotoxin. Prior incubation of the trachea with antiserum to vasoactive intestinal peptide reduced the relaxation. Thus vasoactive intestinal peptide may mediate the nonadrenergic relaxation of tracheal smooth muscle.*

The tracheobronchial tree is innervated by excitatory cholinergic nerves and inhibitory (smooth-muscle relaxant) adrenergic nerves (*1*). The existence of an additional, nonadrenergic, inhibitory system of innervation is demonstrated by relaxation of the airways with electrical field stimulation in vitro (*2*) or with vagal stimulation in vivo (*3*); this relaxation is not inhibited by adrenergic or cholinergic blockade. The physiological significance of this nonadrenergic system varies in different species; it is thought to be the dominant inhibitory influence in airways of guinea pigs, cats, and humans (*2, 3*). Nonadrenergic inhibitory nerves are also believed to be responsible for most of the neural inhibition of the lower esophageal sphincter and other segments of the gastrointestinal tract (*4*).

Some evidence has been presented that adenosine triphosphate or a related nucleotide may be the neurotransmitter for this nervous system in the airways and elsewhere (*5*), but this hypothesis has not been supported by experiments on the cat bronchial tree (*6*). The possibility that the vasoactive intestinal peptide (VIP) may be the responsible mediator is suggested by several observations. This peptide is present in nerve fibers and nerve terminals in the airways (*7*) as well as within the vagus nerve (*8*); it relaxes bronchial smooth muscle independently of adrenergic or cholinergic receptors (*9*); and it fulfills many criteria of a neurotransmitter, including localization in nerve terminals (*10*), release by high potassium ion concentration in the presence of calcium ion (*10*), and binding to specific receptors in the nervous system (*11*). We now report that (i) immunoreactive VIP is released from the guinea pig trachea during the relaxation induced by field stimulation; (ii) this release is abolished by tetrodotoxin, which also blocks the relaxation; and (iii) preliminary incubation with antiserum to VIP reduces the tracheal relaxation.

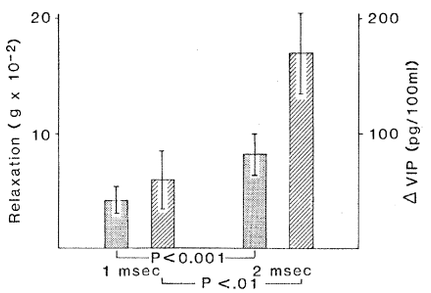

Fig. 1. Tracheal relaxation (stippled bars) and increments of VIP concentration in tracheal bath (hatched bars), induced by electrical field stimulation of isolated guinea pig trachea with 1-msec and 2-msec pulses (N = 18). Values are means ± standard errors. Both relaxation and VIP output at 1-msec stimulations were significantly different from values at control conditions and increased significantly at 2-msec stimulation. Concentrations of VIP in the bath fluid increased to 154 and 438 percent, respectively, of the basal values.

Tracheas from male and female guinea pigs (body weight, 300 to 500 g) were cut spirally into strips about 2 cm long and 2 mm wide. Each tracheal strip was mounted between two platinum electrodes, 6 mm apart, and placed in 5 ml of Krebs solution equilibrated with 95 percent O_2 and 5 percent CO_2 and maintained at 37°C. The bath solution contained propranolol hydrochloride (2 μg/ml) (Ayerst) and atropine sulfate (1 μg/ml) (Lilly), which blocked, respectively, the actions of β-adrenergic (isoproterenol) and cholinergic (acetylcholine) agonists. Baseline tension was adjusted to 1 g initially, and changes in tracheal muscle tension were recorded by an isometric force transducer (Harvard, No. 363) and a two-channel recorder (Beckman Dynograph). After a 1-hour control period, field stimulations were applied by a laboratory stimulator (model 104, American Electronic Laboratories, Philadelphia), with rectangular pulses of 70 V, at a frequency of 20 Hz, and a pulse duration of 1 or 2 msec. Each period of stimulation lasted for 2 minutes, and consecutive stimulations were separated by at least 30 minutes. Krebs solution from the bath was collected before and after stimulation for measurement of immunoreactive VIP by a specific radioimmunoassay (*12*).

Results were analyzed from 18 experiments in which field stimulations caused relaxations of at least 3×10^{-2} g with 2-msec stimulations or 0.5×10^{-2} g with 1-msec stimulations. The relaxation developed gradually after application of the stimulus, reaching a peak within 2 minutes and lasting for 5 to 7 minutes; in these characteristics the relaxations resembled those induced by administration of VIP to the tracheal segment (*9*). The mean relaxation was greater with 2-msec stimulations than with 1-msec stimulations ($P < .001$, Fig. 1). Relaxation was associated with increased immunoreactive VIP in the bath fluid, and the increments in VIP concentrations were also larger with the longer stimulations ($P < .01$, Fig. 1).

In all experiments, the strips were stimulated before and after the addition of tetrodotoxin (1 μg/ml) (Sigma) to the bath. This neurotoxin, which blocks nerve impulse transmission (*13*), abolished tracheal relaxation with both 1-msec and 2-msec stimulations, and there were no significant increases in VIP in the bath fluid. As has also been noted by others (*2*), relaxations elicited by longer (6 to 8 msec) stimulations were not totally blocked by tetrodotoxin.

These results show an association and correlation between the local release of VIP from the tracheal segments and the nonadrenergic, noncholinergic relaxation of these segments elicited by electrical field stimulation. The experiments with tetrodotoxin suggest a neural origin for the released VIP and further link it to the tracheal relaxation. For more direct evidence of a mediator role for VIP in airway relaxation we tested the influence of antiserum to VIP (*14*) on tracheal relaxation in eight experiments. In each of these paired experiments, the antiserum (10 μl in 1 ml of Krebs solution) was in-

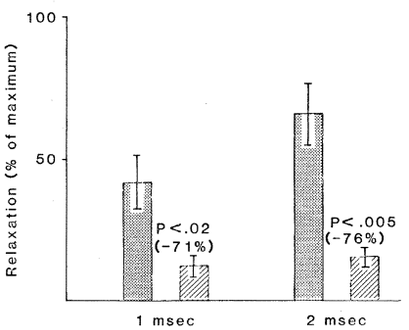

Fig. 2. Tracheal relaxations induced by 1-msec and 2-msec stimulations were greatly reduced after preliminary incubation with antiserum to VIP (hatched bars) in comparison with relaxations of segments of the same tracheas that were not incubated with antiserum [stippled bars (N = 8)]. The magnitude of the relaxation is expressed relative to maximum relaxation produced by a stimulus duration of 6 to 8 msec. Maximum relaxations were comparable in the two groups. Values are means ± standard errors.

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cubated overnight at 4°C with one of the two tracheal segments from each guinea pig; the other segment, serving as control tissue, was incubated under identical conditions, but without the antiserum. The mean relaxation of tracheal strips that had been incubated with antiserum to VIP was lower by 71 percent with 1-msec stimulations and 76 percent with 2-msec stimulations (Fig. 2).

Our results point to VIP as a transmitter of the nonadrenergic inhibitory nervous system in the airways of guinea pigs. Nerves containing VIP are widely distributed in organs with much smooth muscle, especially in sphincters (*15*). This peptide may also mediate enteric inhibitory responses (*16*), including the relaxation of the lower esophageal sphincter that is induced by electrical vagal stimulation.

Identification of the mediator of the nonadrenergic inhibitory system has obvious implications for the neurohumoral regulation of airway smooth muscle tone. Further, it is potentially important for understanding the pathogenesis of bronchial asthma and related bronchospastic disorders. Not only does the nonadrenergic inhibitory system appear to be the principal inhibitory nervous system for human airway smooth muscle (*2*), but a deficiency of this system could explain the hyperreactivity of airways in asthma (*17*). Support for this notion is provided by observations on Hirschsprung's disease, a condition characterized by persistent contraction of the distal bowel and localized absence of autonomic ganglia. In this disease, considered analogous in some respects to bronchial asthma (*17*), the nonadrenergic inhibitory responses are lacking (*18*) and VIP nerves are markedly reduced in affected areas of the gut (*19*).

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*p*H-Sensitive Liposomes: Possible Clinical Implications

Abstract. *When* p*H-sensitive molecules are incorporated into liposomes, drugs can be specifically released from these vesicles by a change of* p*H in the ambient serum. Liposomes containing the* p*H-sensitive lipid palmitoyl homocysteine (PHC) were constructed so that the greatest* p*H differential (6.0 to 7.4) of drug release was obtained near physiological temperature. Such liposomes could be useful clinically if they enable drugs to be targeted to areas of the body in which* p*H is less than physiological, such as primary tumors and metastases or sites of inflammation and infection.*

The use of liposomes as vehicles for preferential delivery of drugs to tumors has been discussed repeatedly (*1*–*4*). Despite the major effort invested in this idea, successful drug targeting is still an elusive goal. Recently, in an attempt to circumvent the targeting problem, it was proposed that local hyperthermia could preferentially release drugs from liposomes in the heated area (*5*, *6*). For this purpose, liposomes were constructed from phospholipid mixtures exhibiting gel-state to liquid crystalline phase transitions at a temperature slightly above body temperature and thus attainable by local hyperthermia.

In two studies in vivo, liposomes containing either radioactive methotrexate (*7*) or *cis*-dichlorodiamine platinum (PDD) (*8*) were injected into tumor-bearing mice. Local heating caused up to fourfold greater drug release and uptake by the tumor than in unheated areas. In addition, PDD specifically released from liposomes by heat produced a greater delay in tumor growth than did either free drug combined with tumor heating or drug containing liposomes but without local heating (*7*, *8*).

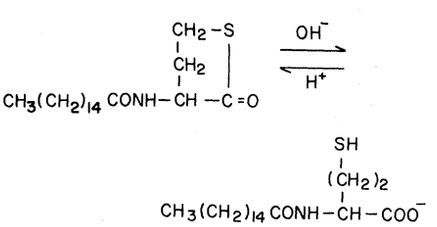

Fig. 1. Equilibrium between the uncharged thiolactone and charged open form of *N*-palmitoyl homocysteine (PHC). The thiolactone form is favored by decreasing *p*H.

The strategy of using local heating to induce preferential release of drugs from liposomes does not effectively address the major problem in human cancer: the metastatic lesions. An alternative approach was suggested by the fact that the interstitial fluids of a number of tumors in humans and animals have an ambient *p*H that is considerably lower than that of normal tissue (*9*–*12*). This difference could be utilized if liposomes could be constructed in a manner such that they would release encapsulated drug when passing through a region of lower *p*H. If microscopic domains of metastases also have a lower *p*H such vesicles could be of particular benefit in chemotherapy. In this report we describe *p*H-sensitive liposomes of possible therapeutic value.

Our approach to *p*H-sensitive lipo-

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