Br. J. Pharmac. (1978), **62**, 367–369

VASOACTIVE INTESTINAL POLYPEPTIDE PROMOTES CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE ACCUMULATION IN GUINEA-PIG TRACHEA

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Cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels in guinea-pig tracheal rings increased on incubation with the vasoactive intestinal peptide (VIP). The effect was potentiated by the addition of theophylline. The results suggest that the tracheo-bronchial relaxant action of VIP may be mediated by stimulation of tracheal adenylyl cyclase.

Introduction The vasoactive intestinal polypeptide (VIP), widely distributed in the gastrointestinal and nervous systems, has multiple biological actions (Said, 1978). Some of these actions are shared by the structurally related peptides, secretin and glucagon. Like these two peptides, VIP has been shown to stimulate the adenylyl cyclase system in several preparations. For VIP this effect has been demonstrated in rat adipocytes (Frandsen & Moody, 1973; Desbuquois, Laudat & Laudat, 1974) rat liver membranes (Desbuquois, Laudat & Laudat, 1973), intestinal mucosa (Schwartz, Kimberg, Sheerin, Field & Said, 1974), isolated enterocytes from rat small intestine (Laburthe, Besson, Hoa & Rosselin, 1977), guinea-pig pancreatic acinar cells (Gardner, Christophe, Robberchet & Conlon, 1977), mouse pancreas homogenates, and mouse isolated islets of Langerhans (Frandsen & Moody, 1977).

Among the demonstrated effects of VIP is its ability to induce relaxation of the smooth muscle of isolated, superfused trachea of guinea-pig (Said, Kitamura, Yoshida, Preskitt & Holden, 1974), and to prevent the bronchoconstrictor action of histamine and prostaglandin $F_{2\alpha}$ in anaesthetized dogs (Hara, Geumei, Chijimatsu & Said, 1975). In this work, we have attempted to characterize the mode of action of VIP on tracheobronchial smooth muscle by measuring VIP-mediated cyclic adenosine 3',5'-monophosphate (cyclic AMP) accumulation in this tissue. A number of agents stimulate the production of cyclic AMP, or inhibit its degradation, in the airways (Andersson, Bergh & Svedmyr, 1972; Inamasu, Shinjo, Iwasawa & Morita, 1974; Murad, 1974; Duncan, Griffin & Solomon, 1975; Ohkubo, Takayanagi & Takagi, 1976; Triner, Velliemoz & Verosky, 1977), and these actions may be related to their ability to produce bronchodilatation (Said *et al.*, 1974; Andersson *et al.*, 1972; Inamasu *et al.*, 1974; Murad, 1974; Duncan *et al.*, 1975; Ohkubo *et al.*, 1976; Triner, *et al.*, 1977). The bronchodilator action of VIP is of special interest in view of the recent demonstration that a VIP-like peptide occurs in normal lung tissue (Said & Mutt, 1977).

Methods We used guinea-pig tracheal rings of an average weight of 11 mg (0.9 mg of protein). The tracheal rings were washed and thermoequilibrated for 30 min at 37°C in 0.5 ml of HEPES buffer (50 mM, pH 7.4) containing 1% human serum albumin and Earle's salt solution. At the end of this preincubation period VIP or other agents (theophylline, isoprenaline) were added, separately and in combinations (Table 1). The tracheal rings were incubated for 5 min at 37°C and the reaction was terminated by the addition of 0.5 ml of 10% perchloric acid. Cyclic AMP was extracted by homogenization with a Polytron homogenizer (Brinkmann). The extracts were neutralized with KOH and purified as previously described (Frandsen & Krishna, 1976), on Bio-Rad AG-1 × 8 columns. The sample content of cyclic AMP was determined by a recently developed radioimmunoassay procedure based on succinylation of the cyclic nucleotide (Frandsen & Krishna, 1976).

Results Only slight, though significant ($P < 0.025$), elevation of the tissue level of cyclic AMP could be detected in the presence of VIP (Table 1) at concentrations producing maximal relaxation of the superfused tracheal preparation (Said *et al.*, 1974). Isoprenaline (8×10^{-6} M) and the phosphodiesterase inhibitor, theophylline, also produced moderate increases in cyclic AMP levels. Theophylline markedly potentiated the effect of both VIP and isoprenaline.

Table 1 Stimulation of cyclic AMP levels in guinea-pig isolated trachea with vasoactive intestinal polypeptide (VIP) and other agents

Additive	*Concentration*	*Cyclic AMP* (pmol mg^{-1} protein)	P[1]
None		10.5 ± 1.0	
VIP	1 μM	17.0 ± 1.5	0.025
Theophylline	5 mM	24.0 ± 1.5	0.005
Isoprenaline	8 μM	32.0 ± 6.5	0.025
VIP plus theophylline	1 μM 5 mM	77.5 ± 4.5	0.001
Isoprenaline plus theophylline	8 μM 5 mM	62.5 ± 3.5	0.001

Results are expressed as mean ± s.e. mean. Number of experiments is shown in parentheses.
[1] *P* value, relative to control measurement (no agent added); calculated by paired comparison, using Student's *t* test.

The highest accumulation rate of cyclic AMP occurred with the VIP-theophylline combination.

Discussion These data show that VIP, especially in combination with theophylline, induces accumulation of cyclic AMP in guinea-pig isolated tracheal rings.

The control values for tracheal cyclic AMP levels are comparable to other published data (Andersson *et al.*, 1972; Inamasu *et al.*, 1974; Murad, 1974; Duncan *et al.*, 1975; Ohkubo *et al.*, 1976; Triner *et al.*, 1977). The small increase observed on incubation with VIP alone is in agreement with earlier observations on isolated adipocytes (Frandsen & Moody, 1973). The potentiation of the effect of VIP on tracheal cyclic AMP levels by theophylline parallels the potentiation reported between these two agents on the relaxation of this tissue (Said *et al.*, 1974). The same finding also suggests that VIP promotes the accumulation of cyclic AMP by stimulating the tracheal adenylyl cyclase system. Although we did not investigate the possible influence of β-receptor blockade in our preparation, the actions of VIP on smooth muscle tissues are known to be independent of these receptors (Piper, Said & Vane, 1970).

Our results suggest that the tracheo-bronchial relaxant effect of VIP may be mediated by stimulation of cyclic AMP production in this tissue. The observations also lend support to the notion that this metabolic action is an important mechanism of bronchodilatation shared by many agents.

This work was supported in part by Center Award HL 14187, from NHLBI, NIH, U.S. Public Health Service.

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(Received November 22, 1977)