

Vasoactive Intestinal Peptide Stimulation of Adenylate Cyclase and Active Electrolyte Secretion in Intestinal Mucosa

CHARLES J. SCHWARTZ, DANIEL V. KIMBERG, HARLAND E. SHEERIN,
MICHAEL FIELD, and SAMI I. SAID

From the Department of Medicine, Harvard Medical School, and the Gastrointestinal Unit of the Department of Medicine, Beth Israel Hospital, Boston 02215, the Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas 75216, and the Veterans Administration Hospital, Dallas, Texas 75235

ABSTRACT Vasoactive intestinal peptide (VIP), originally isolated from hog small intestinal mucosa, has been shown to cause small intestinal secretion. More recently, this peptide has been identified in the plasma and tumors of patients with the so-called "pancreatic cholera" syndrome. In order to explore the possible role of VIP in the pathogenesis of this syndrome, we examined the effects of this peptide and other hormones on the cyclic AMP levels, adenylate cyclase activity, and ion transport in *in vitro* preparations of ileal mucosa. In rabbit ileal mucosa, VIP (20 $\mu\text{g}/\text{ml}$) caused a prompt fivefold increase in cyclic AMP level, whereas nine other hormones, which have been postulated to cause intestinal secretion, failed to exert such an effect. Pentagastrin and glucagon also failed to increase cyclic AMP levels in canine ileal mucosa. An increase in mucosal cyclic AMP levels was observed at a VIP concentration of 0.1 $\mu\text{g}/\text{ml}$ and appeared to be nearly maximal at 2.0 $\mu\text{g}/\text{ml}$. VIP (100 $\mu\text{g}/\text{ml}$) stimulated adenylate cyclase activity in a membrane preparation from rabbit ileal mucosa. Secretin (6.0×10^{-6} M) failed to do so. When added to the serosal side of isolated rabbit ileal mucosa clamped in an Ussing chamber, VIP (2 $\mu\text{g}/\text{ml}$) increased short-circuit current (SCC) and caused net secretion of both Cl and Na. Net Cl secretion exceeded net Na secretion. These effects of VIP on mucosal cyclic AMP metabolism and ion transport are similar to those observed with cholera enterotoxin and certain prostaglandins. VIP was also tested with normal human

ileal mucosa. At a concentration of 2 $\mu\text{g}/\text{ml}$ it caused a fivefold increase in cyclic AMP level and an increase in SCC of the same magnitude as that caused by 5 mM theophylline. Addition of a second 2- $\mu\text{g}/\text{ml}$ dose of VIP and addition of theophylline after VIP produced no further change in SCC. We conclude the VIP stimulates adenylate cyclase and active ion secretion in both rabbit and human ileal mucosa. This may be related to the pathogenesis of diarrhea in patients with the pancreatic cholera syndrome.

INTRODUCTION

Vasoactive intestinal peptide (VIP)¹ was originally isolated from the small intestine of hogs by Said and Mutt (1, 2), and the synthesis of this octacosapeptide has recently been reported by Bodanszky, Klausner, and Said (3). This peptide, related in structure to secretin, glucagon, and gastric inhibitory peptide (GIP), has potent and diverse biological activity. Thus, it has been shown to cause vasodilatation, lowering of arterial blood pressure, increased cardiac output, enhanced myocardial contractility, hyperventilation, smooth muscle relaxation, pancreatic secretion, and glycogenolysis with associated hyperglycemia (1-6). Of special interest with regard to the role which VIP may play in small intestinal function are the observations of Barbezat and Grossman (7) and of Bloom, Polak, and Pearse (8). The former workers (7) demonstrated that intravenously administered VIP caused a striking net secretory response in the

Dr. Kimberg is the recipient of a Research Career Development Award, AM-19377.

Received for publication 11 January 1974 and in revised form 15 April 1974.

¹ Abbreviations used in this paper: GIP, gastric inhibitory peptide; KRB, Krebs-Ringer bicarbonate solution; SCC, short-circuit current; VIP, vasoactive intestinal peptide.

small intestine of dogs with Thiry-Vella loops and a decrease in histamine-stimulated acid secretion in dogs with Heidenhain pouches. More recently, Bloom and his coworkers (8) reported elevated plasma levels and/or a high tumor content of VIP by specific radioimmunoassay in six patients with watery diarrhea associated with the so-called pancreatic cholera syndrome (9-13).

Both cholera enterotoxin and certain prostaglandins stimulate adenylate cyclase in small intestinal mucosal cells (14, 15), which thus increases the cellular content of cyclic AMP (14, 16, 17). Furthermore, there is now good reason to believe that the diarrhea seen in cholera (18), in association with the administration of prostaglandins (19-21) or in patients with certain prostaglandin-secreting tumors (22, 23), is due to cyclic AMP-mediated stimulation of water and electrolyte secretion, inhibition of active absorption thus unmasking an active secretory process, or some combination of both events (24-29). In view of the known effects of VIP on small intestinal secretion (7), its recent implication as a potential secretagogue in the pancreatic cholera syndrome (8), and the recent demonstration that this peptide can stimulate adenylate cyclase activity in liver (30) and in fat cell membranes (30, 31), the present studies were undertaken to examine the effects of VIP and certain other hormones on intestinal cyclic AMP metabolism and ion transport in isolated rabbit and dog ileum. In addition, the effects of VIP on human mucosa obtained from one patient were also studied.

METHODS

Cyclic AMP measurements. New Zealand white male rabbits (2-3.5 kg) and male mongrel dogs (10-12 kg) were used in the present study. Rabbits were sacrificed by a blow to the neck, while the dogs were anesthetized with intravenous pentobarbital, intubated, and then maintained in stage IV anesthesia with halothane until the end of the procedure at which time they too were sacrificed. The terminal ileum was quickly removed, opened longitudinally, and rinsed in ice-cold Krebs-Ringer bicarbonate solution (KRB) gassed with 95% O₂-5% CO₂, as previously described (17). The ileum was stripped of muscularis and 3-6 segments of mucosa, each approximately 50 mg in wet weight, were added to 50-ml Erlenmeyer flasks containing 7.5 ml of KRB. In certain experiments in which a larger number of segments were incubated in the same flasks, the volume of buffer was increased accordingly. During a 40-min preincubation at 37°C, the flasks were agitated at 80 rpm while the tissue was continuously gassed in an atmosphere of 95% O₂-5% CO₂. After preincubation, various hormones or appropriate solvents were added and the flasks were allowed to incubate for varying times between 1 and 40 min. Tissues were prepared for cyclic AMP measurements as previously described (17). At least three segments of tissue were used to test each variable in each separate experiment. Furthermore, duplicate determinations of cyclic AMP were performed on each sample by employing the protein-kinase binding assay developed by Gilman (32) and modified as previously described (17). Each hormone was tested in

vitro in this manner with rabbit ileal mucosa. In addition, gastrin, glucagon, and the combination of the two were tested with canine ileal mucosa.

Adenylate cyclase assay. Membranes were prepared from a homogenate of ileal mucosa as previously described (14). The final pellet was resuspended with a glass rod in a sufficient volume of 10⁻² M glycylglycine buffer at pH 7.8, containing 10⁻³ M MgSO₄, such that each milliliter contained between 7.0 and 8.0 mg of protein as determined by the method of Lowry, Rosebrough, Farr, and Randall (33). Adenylate cyclase activity was measured by a modification of the procedure described by Krishna, Weiss, and Brodie (34). Incubations were initiated by the addition of 20 μl of homogenate containing between 140-160 μg protein to 30 μl of medium in a reaction mixture which contained 50 mM Tris-HCl buffer at pH 7.4, 5 mM MgCl₂, 10 mM caffeine, 2.0 mM ATP, 2 μCi of [8-¹⁴C]ATP (New England Nuclear, Boston, Mass.), and an ATP-regenerating system consisting of 10 mM phosphoenolpyruvate and 12.5 μg of pyruvate kinase. Base-line activity with appropriate solvent controls, as well as activity in the presence of VIP, secretin, and NaF, were assessed. Incubations were performed for 5 min at 37°C. Preliminary experiments indicated that, under the conditions employed, enzyme activity was linear with time and protein concentration. Just before the termination of the reaction, 0.2 ml of a solution containing 60 μg of cyclic AMP and 0.25 μCi of [³H]cyclic AMP was added to each tube. The reaction was terminated by immersing each tube in a boiling water bath for 3 min, and the incubation medium was then passed over a 0.5 × 3.0-cm column containing Dowex 50 W X 4 resin (Dow Chemical Co., Midland, Mich.), hydrogen form, 200-400 mesh (Calbiochem, San Diego, Calif.). Subsequent steps have been previously described (14). All assays were performed in triplicate.

Measurements of short-circuit current (SCC) and ion fluxes. 20-30-cm segments of distal rabbit ileum were quickly excised, opened longitudinally, and rinsed in KRB. Four 1-2-cm sections were stripped of muscularis and mounted as flat sheets in Ussing chambers as previously described (35). Electric potential difference, SCC, and DC conductance were monitored as previously described (35). Unidirectional steady state mucosa-to-serosa and serosa-to-mucosa fluxes of Na and Cl were determined on paired tissues by using ²²Na and ³⁶Cl (both New England Nuclear). In earlier experiments, Na fluxes alone were determined; in later experiments, Na and Cl fluxes were determined simultaneously. Radioisotopes were added 30 min after mounting tissues. 20 min thereafter, VIP (2 μg/ml) was added to the serosal reservoirs of one pair of tissues, the other pair served as control. Tissues were then maintained in the short-circuited state, except for occasional measurements of open circuit potential difference. Fluxes were calculated as previously described (35) from reservoir samples taken at 10-min intervals for 40 min. Results for the three flux periods (10-40 min post-VIP) were then averaged. In some experiments, after the final sample had been taken, theophylline (5 μmol/ml) was added to the serosal reservoirs, and the resulting changes in SCC were recorded.

Studies on human ileum. Distal ileum was removed from near the proximal resection margin of a 50-cm segment of terminal ileum obtained from a 36-yr-old female patient with regional enteritis involving terminal ileum. The ileum thus obtained was kept in ice-cold saline until ready for incubation (about 20 min). In order to determine the effect

of VIP on cyclic AMP level, mucosa was separated from the underlying muscle, and segments were incubated in Erlenmeyer flasks exactly as described above for rabbit ileum. Three segments of tissue were incubated in each of four flasks and, after a 40-min preincubation period, VIP (2 $\mu\text{g}/\text{ml}$) was added to two flasks. 10 min later all tissues were removed, and cyclic AMP levels were determined.

Sheets of mucosa, removed by scraping with the edge of a glass microscope slide, were mounted in three Ussing chambers and bathed on both sides with 10 ml of KRB. Electric potential difference, resistance, and SCC were determined as previously described (35). Additions of VIP and theophylline were made to two of the three chambers 50–90 min after mounting the tissues. Glucose (10 $\mu\text{mol}/\text{ml}$) was added to the luminal bathing solution of all three chambers about 2 h after mounting. Glucose produced a rapid increase in SCC (mean increment, 107 $\mu\text{A}/\text{cm}^2$; range 93–128) which indicated that the mechanism for glucose-coupled Na transport was normal in the mucosal sheets used for these experiments (36). After terminating the experiments, tissues were removed from the chambers, placed in 10% formalin, and processed for microscopic examination. Hematoxylin and eosin-stained sections revealed normal ileal mucosa.

Statistical analyses. All statistical analyses were done by Student's *t* test for either paired or unpaired variates.

Materials. VIP, prepared at the Karolinska Institute as described by Said and Mutt (1, 2), was dissolved in 0.9% NaCl and employed at the concentrations noted in the Tables, Fig., or their legends. GIP was a gift of Dr. John C. Brown of the Department of Physiology, University of British Columbia. Pentagastrin (Calbiochem), 5-hydroxytryptamine (Sigma Chemical Co., St. Louis, Mo.), bradykinin triacetate (Sigma Chemical Co.), crystalline glucagon (Sigma Chemical Co.), carbamylcholine chloride (Sigma Chemical Co.), and vasopressin (Pitressin, Parke, Davis and Co., Detroit, Mich.), were employed at concentrations noted in Table II. Synthetic salmon calcitonin was a gift of Dr. John T. Potts, Jr. of the Department of Medicine at Harvard Medical School. Synthetic secretin (Pansecrin,

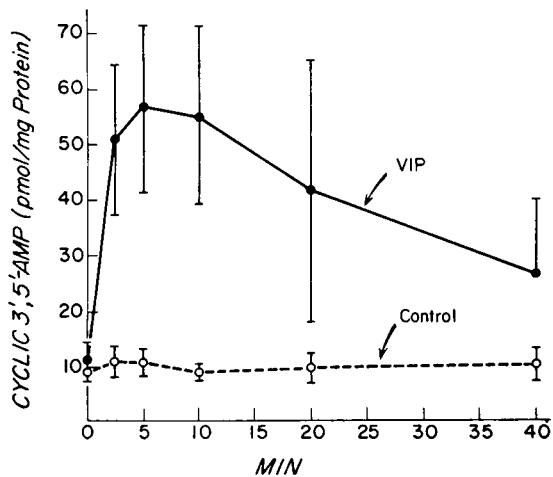


FIGURE 1 Effects of VIP (20 $\mu\text{g}/\text{ml}$) on cyclic AMP levels in rabbit ileal mucosa expressed as a function of time. Each point is the mean \pm 1 SEM (brackets) for five complete and separate experiments performed as described in Methods.

TABLE I
Effect of Varying Concentrations of VIP on Cyclic AMP Levels in Rabbit Ileal Mucosa

VIP $\mu\text{g}/\text{ml}$	Cyclic 3', 5'-AMP		
	Expt 1	Expt 2	Expt 3
	<i>pmol/mg protein</i>		
None	8.53	7.38	10.92
0.001	12.12	4.15	8.06
0.010	9.11	7.57	7.69
0.100	22.36	14.83	—
0.500	58.28	50.48	32.17
2.000	86.25	146.00	85.70
10.000	97.06	155.49	104.90

E. R. Squibb and Sons, New York), a gift of Dr. M. Ondetti of the Squibb Institute, was employed in experiments in which cyclic AMP levels in ileal mucosa were measured. Synthetic secretin (Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, N. Y.) was tested in the adenylate cyclase assay system. Appropriate solvent controls were employed for each hormone.

RESULTS

Effects of VIP and other hormones on cyclic AMP levels. VIP was found to cause a striking increase in rabbit ileal mucosal cyclic AMP levels in five separate experiments (Fig. 1). Thus, in the presence of 20 $\mu\text{g}/\text{ml}$ of VIP/ml, there was a fivefold increase in cyclic AMP levels at 2, 5, and 10 min ($P < 0.05$), with a gradual return towards control values during the ensuing 30 min. Neither the 20- nor 40-min cyclic AMP levels in the presence of VIP are significantly different from the corresponding control levels. Furthermore, the 40-min levels in the presence of VIP are lower than those at 5 min ($P < 0.05$) and 10 min ($P < 0.02$). The large standard errors in the presence of VIP are probably related to variations in the tissues from the animals used in the five separate experiments.

Table I demonstrates the results of three experiments in which the effects of varying concentrations of VIP on rabbit ileal mucosal cyclic AMP levels were assessed after a 5-min incubation. An effect was present at 0.1 $\mu\text{g}/\text{ml}$, and the effect was nearly maximal at 2.0 $\mu\text{g}/\text{ml}$.

A number of hormones which have been found to cause small intestinal secretion, which have been suggested as potential secretagogues in the pancreatic cholera syndrome or which are known to elevate cyclic AMP levels in nonintestinal tissues failed to increase the level of this nucleotide in ileal mucosa (Table II). Neither glucagon nor pentagastrin, alone or in combination, led to a change in cyclic AMP levels in rabbit ileal mucosa after 5 and 10 min of incubation. Since Barbezat and Grossman (7, 37, 38) have shown that

TABLE II

Effect of Potential Secretagogues on Cyclic AMP Levels in Rabbit Ileal Mucosa

Potential secretagogues	Concn	No.	Time, min.			
			1	2	5	10
Cyclic 3', 5'-AMP						
pmol/mg protein						
Pentagastrin	5.0×10^{-5} M	4	—	—	4.93 ± 1.35	5.55 ± 1.40
Control	—	—	—	—	4.61 ± 1.50	4.47 ± 1.26
Glucagon	1.2×10^{-6} M	3	—	—	3.62 ± 0.55	3.56 ± 0.60
Control	—	—	—	—	3.11 ± 0.12	3.22 ± 0.27
Pentagastrin and glucagon	Same as above	4	—	—	4.54 ± 0.74	3.87 ± 0.81
Control	—	—	—	—	3.96 ± 0.85	3.87 ± 0.67
Calcitonin	2.9×10^{-6} M	6	—	—	7.10 ± 1.61	5.45 ± 0.78
Control	—	—	—	—	5.42 ± 1.05	5.00 ± 0.82
Secretin	7.8×10^{-7} M	3	—	5.90 ± 1.13	6.43 ± 0.54	5.43 ± 0.26
Control	—	—	—	6.50 ± 0.92	5.47 ± 0.23	5.40 ± 0.66
Carbacol	1.0×10^{-4} M	3	—	8.20 ± 1.81	7.53 ± 1.70	7.67 ± 1.57
Control	—	—	—	8.37 ± 0.92	7.10 ± 0.68	7.13 ± 1.21
GIP	2.4×10^{-6} M	3	—	8.43 ± 0.23	8.43 ± 0.28	7.77 ± 0.56
Control	—	—	—	8.93 ± 0.17	8.63 ± 0.32	8.07 ± 0.84
Serotonin	5.0×10^{-5} M	3	7.48 ± 0.43	7.25 ± 0.86	7.96 ± 1.19	6.85 ± 0.18
Control	—	—	7.14 ± 0.58	7.42 ± 0.92	8.55 ± 1.28	9.20 ± 2.40
Bradykinin	5.0×10^{-5} M	6	11.22 ± 1.27	—	—	—
Control	—	—	8.42 ± 1.07	—	—	—
Vasopressin	100 mU/ml	4	10.22 ± 1.74	—	8.74 ± 1.40	—
Control	—	—	10.28 ± 2.21	—	—	—

Values are means \pm 1 SEM for number of animals. Potential secretagogues or solvents added at zero time.

these peptides, and more especially the two combined (38), are effective in inducing a net secretory state in canine small intestine in vivo, the effects of these hormones on mucosal cyclic AMP metabolism was also assessed with material from this species. Once again, individually or in combination, glucagon and pentagas-

trin failed to alter cyclic AMP levels measured after 1, 5, and 10 min of incubation (Table III).

Effects of VIP and secretin on adenylate cyclase activity. VIP and secretin were added to membranes prepared from rabbit ileum. As noted in Fig. 2, concentrations of VIP as low as 0.5 μ g/ml caused significant

TABLE III

Effect of Pentagastrin and Glucagon on Cyclic AMP Levels in Canine Ileal Mucosa

Peptide	Concn	Time, min.	Cyclic 3', 5'-AMP*		
			1	5	10
pmol/mg protein					
Pentagastrin	5.0×10^{-5} M		6.46 ± 0.60	5.82 ± 0.16	7.11 ± 1.02
Glucagon	1.2×10^{-6} M		6.33 ± 0.32	6.22 ± 0.28	7.07 ± 1.12
Pentagastrin and glucagon	Same as above		7.00 ± 1.07	7.03 ± 0.90	7.28 ± 1.07
Control	—		6.54 ± 0.63	6.44 ± 0.42	7.61 ± 1.77

Values are means \pm 1 SEM for three experiments. Peptides or solvents added at zero time.

* No significant difference between groups at 1, 5, and 10 min ($P > 0.05$).

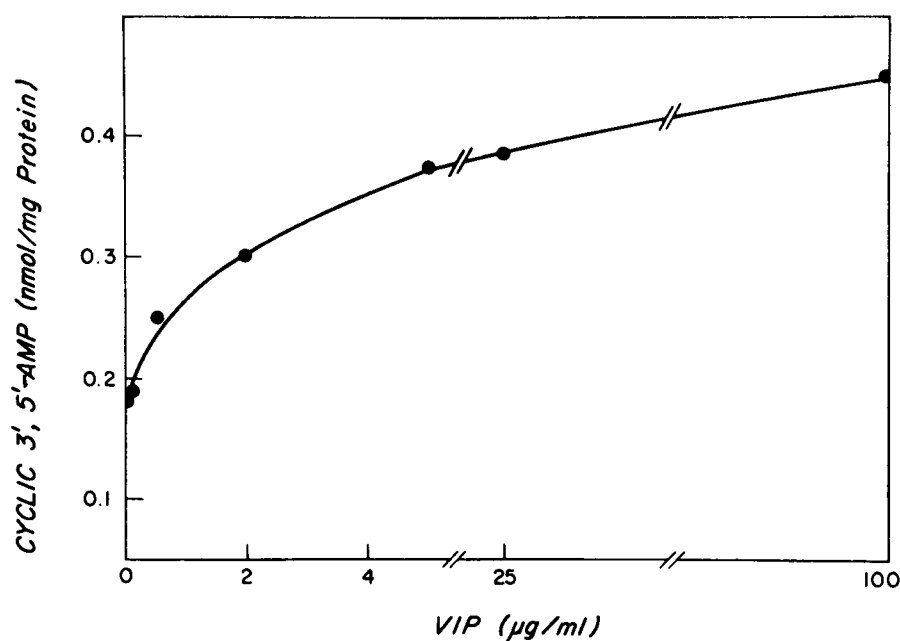


FIGURE 2 Effects of varying concentrations of VIP on adenylate cyclase activity in rabbit ileal mucosa. Each point is the mean for three separate experiments performed as described in Methods.

stimulation of adenylate cyclase ($P < 0.05$), and a near maximal effect was obtained at a concentration of $5.0 \mu\text{g/ml}$. These results are consistent with those in Table I in which the effects of varying concentrations of VIP on ileal cyclic AMP levels are reported.

In two separate groups of experiments, the effects on cyclase activity of VIP, secretin, and NaF were compared (Table IV). Whereas VIP doubled the base-line adenylate cyclase activity ($P < 0.01$), secretin was ineffective. Cyclase activity in the presence of 10^{-2} M NaF

was greater than in VIP-containing incubations ($P < 0.05$).

Effects of VIP on SCC and fluxes of Na and Cl. In view of the striking effects of VIP on cyclic AMP metabolism in ileal mucosa, and in view of the previously described effects of cyclic AMP (39), prostaglandins (14, 17, 25–29), and cholera enterotoxin (24, 25) on ion transport and the electrical properties of this tissue, studies were undertaken to evaluate the effects of VIP on SCC and ion fluxes.

TABLE IV
Effects of VIP, Secretin, and NaF on Adenylate Cyclase Activity in Membranes from Rabbit Ileal Mucosa

	Cyclic 3', 5'-AMP		
	Control	VIP, $100 \mu\text{g/ml}$	NaF, 10^{-2} M
	<i>nmol/mg protein/5 min</i>		
Expt 1*	0.11 ± 0.02	$0.23 \pm 0.04 \ddagger$	$0.37 \pm 0.07 \S$
			Secretin, $5.6 \times 10^{-5} \text{ M}$
Expt 2	0.13 ± 0.06	$0.28 \pm 0.10 \P$	$0.13 \pm 0.04^{**}$

Values are means ± 1 SEM for experiments performed as described in Methods.

* Six experiments.

‡ VIP > control, $P < 0.01$.

§ NaF > control and VIP, $P < 0.01$ and < 0.05 , respectively.

|| Three experiments.

¶ VIP > control, $P < 0.05$.

** Secretin vs. control, not significant

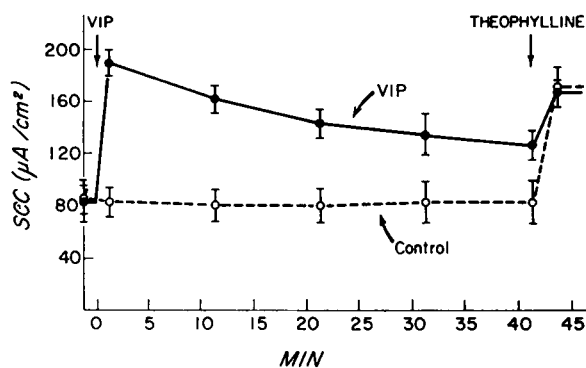


FIGURE 3 Effects of VIP ($2 \mu\text{g/ml}$) on SCC and SCC response to theophylline ($5 \mu\text{mol/ml}$). These compounds were added to the serosal bathing solution at the times indicated by the vertical arrows. Each point is the mean ± 1 SEM (brackets) for six rabbits. Results were obtained in some of the experiments for which the flux data are given in Table V.

Effects of VIP on SCC and SCC response to theophylline are shown in Fig. 3. After addition of the peptide (2 $\mu\text{g}/\text{ml}$) to the serosal reservoir, SCC increased rapidly, reaching a peak in about 1 min. The SCC declined slowly thereafter but remained significantly greater than the control level for 30 min ($P < 0.05$). Addition of theophylline 40 min after VIP caused a considerably smaller increase in SCC in the VIP-treated tissues than in controls ($P < 0.01$). The SCC response to VIP is similar to that previously observed for cyclic AMP (39) and the prostaglandins (29). Blunting of the SCC response to theophylline has previously been noted in studies with cholera toxin (24) and prostaglandins (14).

Effects of VIP on Na and Cl fluxes across short-circuited ileal mucosa are shown in Table V. VIP increased SCC and decreased net Na and Cl fluxes, causing net secretory fluxes of 1.2 and 3.8 $\mu\text{eq}/\text{h cm}^2$, respectively. The changes in both net fluxes were associated with reductions in the mucosa-to-serosa unidirectional fluxes. The serosa-to-mucosa unidirectional fluxes did not change significantly. The magnitudes of net flux changes produced by VIP are similar to those previously observed in this laboratory for cyclic AMP (39), theophylline (39), and cholera enterotoxin (24). The data in Table V also indicate that VIP did not significantly increase residual ion flux (average SCC - net Na flux + net Cl flux), which averaged 1.4 $\mu\text{eq}/\text{h cm}^2$ in controls and 2.0 in VIP-treated tissues. The residual flux has been shown to be an approximate measure of net HCO_3^- secretion in the ileum (40). In this respect also, the present results for VIP are similar to those previously reported for cyclic AMP (39), theophylline (39), dibutyryl cyclic AMP (40), and cholera enterotoxin (24).

Effects of VIP on cyclic AMP level and SCC of human ileal mucosa. In order to examine the effect of VIP on human small intestine, normal distal ileum was obtained from the operating room, and ileal mucosa was incubated in vitro as described in Methods. Cyclic AMP levels (mean \pm 1 SEM) for six VIP-treated and six control tissues (all from the same patient) were 23.6 ± 2.2 and 5.1 ± 0.36 pmol/mg protein, respectively. Thus, VIP produced a fivefold increase in cyclic AMP ($P < 0.001$) within 10 min of addition.

As shown in Fig. 4, addition of VIP (2 $\mu\text{g}/\text{ml}$) to the serosal bathing solution produced a $62 \mu\text{A}/\text{cm}^2$ increase in SCC, which was almost identical in magnitude to that produced by addition of theophylline to another chamber ($64 \mu\text{A}/\text{cm}^2$). The effect of this dose of VIP on SCC was apparently maximal, since neither a second addition of VIP nor subsequent addition of theophylline caused any further increase in SCC. VIP (10 $\mu\text{g}/\text{ml}$) was also added to the luminal bathing solution of a third chamber. No change in SCC resulted, which suggests that relevant VIP receptors are not present on the luminal surface of the ileum.

DISCUSSION

A number of hormones have been reported to inhibit absorption or cause net secretion of water and electrolytes in the small intestine. These hormones include secretin (41-43), gastrin (7, 41, 44, 45), glucagon (7, 37), the combination of gastrin and glucagon (38, 42), cholecystokinin (41, 46), GIP (7), VIP (7), prostaglandins (14, 17, 26-29), thyrocalcitonin (47), Pitressin (48), and acetylcholine (49-51). Only in the case of the prostaglandins has the mechanism underlying the secretory response been previously elucidated, and in this instance, stimulation of adenylate cyclase with a re-

TABLE V
Effects of VIP on Ion Fluxes and Conductance

	Ion fluxes				Conductance
	M \rightarrow S*	S \rightarrow M*	Net	SCC	
	$\mu\text{eq}/\text{h}\cdot\text{cm}^2$				$\text{m}\Omega/\text{cm}^2$
Na Fluxes					
Control (10)	11.6 ± 0.88	10.2 ± 0.85	1.4 ± 0.73	2.4 ± 0.33	24.4 ± 1.4
VIP (9)	8.3 ± 0.59	9.4 ± 0.50	-1.2 ± 0.39	4.8 ± 0.40	21.8 ± 1.7
P	<0.01	NS	<0.01	<0.001	NS
Cl Fluxes					
Control (5)	9.8 ± 1.3	9.4 ± 1.3	0.5 ± 0.30	2.3 ± 0.49	22.5 ± 1.9
VIP (5)	6.6 ± 0.54	10.4 ± 1.0	-3.8 ± 0.72	4.3 ± 0.47	18.4 ± 1.8
P	<0.05	NS	<0.001	<0.02	NS

Values are means \pm 1 SEM. Number of animals are in parentheses. Control and VIP data are not paired, but in most instances, flux measurements for both conditions were obtained on tissues from the same animals.

* M, mucosa; S, serosa.

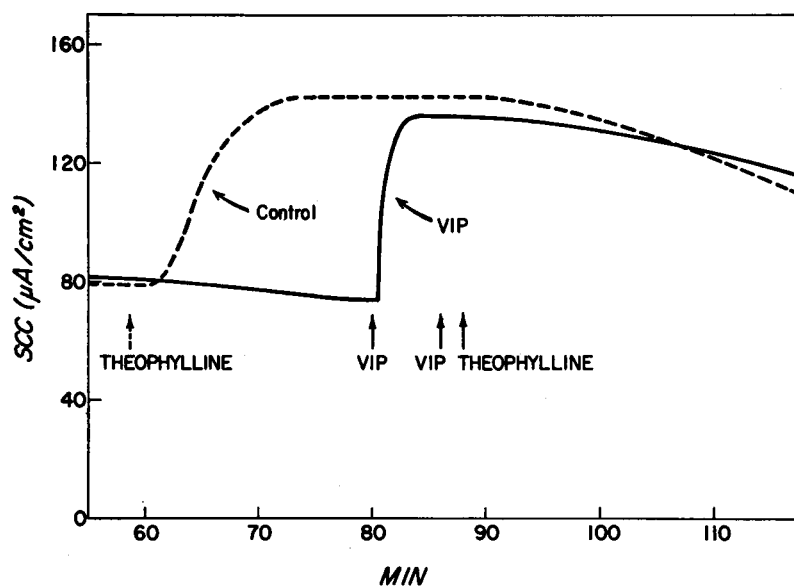


FIGURE 4 Effects of VIP and theophylline on SCC of human ileal mucosa. Times shown are the minutes after tissues were mounted in chambers. Compounds were added to the serosal bathing solutions at the times indicated by the vertical arrows. Theophylline alone ($5 \mu\text{mol/ml}$) was added to one tissue ("Control") and two additions of VIP ($2 \mu\text{g/ml}$ each) and then theophylline ($5 \mu\text{mol/ml}$) were added to the other tissue ("VIP"). The SCC response to VIP had a shorter lag period and a faster half-time of rise (0.5 min each) than did the SCC response to theophylline (2 and 4 min, respectively). The VIP-treated tissue showed no further increase in SCC upon addition of theophylline. The electrical resistance of the control mucosa was $27 \Omega \cdot \text{cm}^2$ immediately before and $30 \Omega \cdot \text{cm}^2$ 15 min after addition of theophylline, and the electrical resistance of the VIP-treated mucosa was $26 \Omega \cdot \text{cm}^2$ immediately before and $30 \Omega \cdot \text{cm}^2$ 6 min after addition of VIP.

sultant increase in mucosal cyclic AMP levels appears to be involved (14, 17). The results of the present study indicate that the known effects of VIP on canine small intestinal secretion (7) may likewise be mediated by a cyclic AMP-dependent mechanism. Thus, VIP produces effects on cyclic AMP metabolism, Na and Cl fluxes, and SCC in rabbit ileum which are similar to those seen with diarrheogenic agents such as cholera enterotoxin and certain prostaglandins (14-18, 24-29, 39, 40). Under the conditions employed in the present study, VIP was the only one of 10 potential secretagogues to increase cyclic AMP levels in ileal mucosa. The results of the present experiments suggest, therefore, that the other hormones studied may effect intestinal secretion by a mechanism which *may* not involve a direct interaction with intestinal mucosal adenylate cyclase.

VIP, glucagon, secretin, and GIP have been shown to bear certain structural similarities (2, 3, 52), and it is therefore, not surprising that they have certain biologic effects in common. Each peptide can inhibit gastric acid secretion (7, 53-56) and, at least in the intact animal, each may be capable of inducing intestinal secretion (7, 37, 41-43). Like glucagon, VIP stimulates hepatic glycogenolysis (5) and myocardial contraction (6); like secretin, it stimulates pancreatic exocrine secretion (2);

and like both glucagon and secretin, VIP stimulates lipolysis (30, 31).

As is the case with the small intestinal mucosa, it is likely that the effects of VIP on the liver and adipose tissue, and perhaps on the pancreas and heart, are cyclic AMP mediated. Thus, glucagon (57-59), secretin (30), and VIP (30) stimulate adenylate cyclase activity in particulate fractions from liver, and all three hormones also stimulate adenylate cyclase in fat cell membranes (30, 31, 60-62). Effects of VIP on myocardial and pancreatic adenylate cyclase have not been reported; the former is activated by glucagon (63, 64) and the latter by secretin (65, 66). There is reason to believe that VIP and glucagon may interact with separate hormone receptor sites in liver and adipose tissue (30, 67), while VIP and secretin may activate cyclase via a common receptor in these tissues (30). Repeated attempts in this study and in previous work (14) to demonstrate an effect of either secretin or glucagon on intestinal mucosal cyclic AMP levels or on adenylate cyclase activity in ileal mucosal cell membranes have been unrewarding. These results suggest that the small intestinal mucosal cyclase complex lacks receptors with significant affinities for these peptides.

The role of endogenous VIP in regulating normal

intestinal and extraintestinal functions remains to be established, but it is clear that in vitro and in vivo additions of this peptide can exert a broad range of biologic effects. It is also clear that VIP, which has been identified in the tumors and/or plasma of certain patients with the pancreatic cholera syndrome (8), has many of the credentials which would be required for it to play a role as a secretagogue in this syndrome. The results of the present study, which included experiments on human as well as rabbit ileal mucosa, suggests that the diarrhea seen in patients with VIP-bearing tumors may be due to an interaction between this peptide and small intestinal mucosal cell adenylate cyclase. If indeed this proves to be the case, then the term, "pancreatic cholera," first used by Matsumoto, Peter, Schultze, Hakim, and Franck in 1966 (11) may be even more appropriate 8 yr later.

ACKNOWLEDGMENTS

This study was supported in part by Grants AM-13696 and AM-05114 from the National Institutes of Health, Department of Health, Education and Welfare, and by the United States-Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (Grant AI-09029).

REFERENCES

- Said, S. I., and V. Mutt. 1970. Polypeptide with broad biological activity: isolation from small intestine. *Science (Wash. D. C.)*. **169**: 1217-1218.
- Said, S. I., and V. Mutt. 1972. Isolation from porcine-intestinal wall of a vasoactive octacosapeptide related to secretin and to glucagon. *Eur. J. Biochem.* **28**: 199-204.
- Bodanszky, M., Y. S. Klausner, and S. I. Said. 1973. Biological activities of synthetic peptides corresponding to fragments of and to the entire sequence of vasoactive intestinal peptide. *Proc. Natl. Acad. Sci. U. S. A.* **70**: 382-384.
- Piper, P. J., S. I. Said, and J. R. Vane. 1970. Effects on smooth muscle preparations of unidentified vasoactive peptides from intestine and lung. *Nature (Lond.)*. **225**: 1144-1145.
- Kerins, C., and S. I. Said. 1973. Hyperglycemic and glycogenolytic effects of vasoactive intestinal polypeptide. *Proc. Soc. Exp. Biol. Med.* **142**: 1014-1017.
- Said, S. I., L. P. Boshier, J. A. Spath, and H. A. Kontos. 1972. Positive inotropic action of newly isolated vasoactive intestinal polypeptide (VIP). *Clin. Res.* **20**: 29 (Abstr.).
- Barbezat, G. O., and M. I. Grossman. 1971. Intestinal secretion: stimulation by peptides. *Science (Wash. D. C.)*. **174**: 422-423.
- Bloom, S. R., J. M. Polak, and A. G. E. Pearse. 1973. Vasoactive intestinal peptide and watery-diarrhea syndrome. *Lancet*. **2**: 14-16.
- Verner, J. V., and A. B. Morrison. 1958. Islet cell tumor and a syndrome of refractory watery diarrhea and hypokalemia. *Am. J. Med.* **25**: 374-380.
- Marks, I. N., S. Bank, and J. H. Louw. 1967. Islet cell tumor of the pancreas with reversible watery diarrhea and achlorhydria. *Gastroenterology*. **52**: 695-708.
- Matsumoto, K. K., J. B. Peter, R. G. Schultze, A. A. Hakim, and P. T. Franck. 1966. Watery diarrhea and hypokalemia associated with pancreatic islet cell adenoma. *Gastroenterology*. **50**: 231-242.
- Kraft, A. R., R. K. Tompkins, and R. M. Zollinger. 1970. Recognition and management of the diarrheal syndrome caused by nonbeta islet cell tumors of the pancreas. *Am. J. Surg.* **119**: 163-170.
- Schein, P. S., R. A. DeLellis, C. R. Kahn, P. Gorden, and A. R. Kraft. 1973. Islet cell tumors: current concepts and management. *Ann. Intern. Med.* **79**: 239-257.
- Kimberg, D. V., M. Field, J. Johnson, A. Henderson, and E. Gershon. 1971. Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandins. *J. Clin. Invest.* **50**: 1218-1230.
- Sharp, G. W. G., and S. Hynie. 1971. Stimulation of intestinal adenyl cyclase by cholera toxin. *Nature (Lond.)*. **229**: 266-269.
- Schafer, D. E., W. D. Lust, B. Sircar, and N. D. Goldberg. 1970. Elevated concentration of adenosine 3':5' cyclic monophosphate in intestinal mucosa after treatment with cholera toxin. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 851-856.
- Kimberg, D. V., M. Field, E. Gershon, and A. Henderson. 1974. Effects of prostaglandins and cholera enterotoxin on intestinal mucosal cyclic AMP accumulation: evidence against an essential role for prostaglandins in the action of toxin. *J. Clin. Invest.* **53**: 941-949.
- Finkelstein, R. A. 1973. Cholera. *Chemical Rubber Company Crit. Rev. Microbiol.* **2**: 553-623.
- Horton, E. W., I. H. M. Main, C. J. Thompson, and P. M. Wright. 1968. Effect of orally administered prostaglandin E₁ on gastric secretion and gastrointestinal motility in man. *Gut*. **9**: 655-658.
- Misiewicz, J. J., S. L. Waller, E. W. Horton, and N. Kiley. 1969. Effect of oral prostaglandin E₁ on intestinal transit in man. *Lancet*. **1**: 648-651.
- Karim, S. M. M. 1970. Therapeutic abortion using prostaglandin F_{2α}. *Lancet*. **1**: 157-159.
- Sandler, M. 1968. Prostaglandins in amine-peptide-secreting tumours. *Lancet*. **2**: 1053-1054.
- Williams, E. D., S. M. M. Karim, and M. Sandler. 1968. Prostaglandin secretion by medullary carcinoma of the thyroid. A possible cause of the associated diarrhea. *Lancet*. **1**: 22-23.
- Field, M., D. Fromm, Q. Al-Awqati, and W. B. Greenough, III. 1972. Effect of cholera enterotoxin on ion transport across isolated ileal mucosa. *J. Clin. Invest.* **51**: 796-804.
- Field, M. 1974. Intestinal secretion. *Gastroenterology*. **66**: 1063-1084.
- Pierce, N. F., C. C. J. Carpenter, Jr., H. L. Elliott, and W. B. Greenough, III. 1971. Effects of prostaglandins, theophylline, and cholera exotoxin upon transmucosal water and electrolyte movement in the canine jejunum. *Gastroenterology*. **60**: 22-32.
- Cummings, J. H., A. Newman, G. J. Milton-Thompson, J. A. Billings, and J. J. Misiewicz. 1973. Effect of intravenous prostaglandin F_{2α} on small intestinal function in man. *Nature (Lond.)*. **243**: 169-171.
- Matuchansky, C., and J. J. Bernier. 1973. Effect of prostaglandin E₁ on glucose, water, and electrolyte absorption in the human jejunum. *Gastroenterology*. **64**: 1111-1118.
- Al-Awqati, Q., and W. B. Greenough, III. 1972. Prostaglandins inhibit intestinal sodium transport. *Nat. New Biol.* **238**: 26-27.

30. Desbuquois, B., M. H. Laudat, and Ph. Laudat. 1973. Vasoactive intestinal polypeptide and glucagon: stimulation of adenylate cyclase activity via distinct receptors in liver and fat cell membranes. *Biochem. Biophys. Res. Commun.* **53**: 1187-1194.
31. Frandsen, E. K., and A. J. Moody. 1973. Lipolytic action of a newly isolated vasoactive intestinal polypeptide. *Horm. Metab. Res.* **5**: 196-199.
32. Gilman, A. G. 1970. A protein binding assay for adenosine 3':5'-cyclic monophosphate. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 305-312.
33. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
34. Krishna, G. B., B. Weiss, and B. B. Brodie. 1968. A simple sensitive method for the assay of adenylyl cyclase. *J. Pharmacol. Exp. Ther.* **163**: 379-385.
35. Field, M., D. Fromm, and I. McColl. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. *Am. J. Physiol.* **220**: 1388-1396.
36. Al-Awqati, Q., J. L. Cameron, and W. B. Greenough, III. 1973. Electrolyte transport in human ileum: Effect of purified cholera exotoxin. *Am. J. Physiol.* **224**: 818-823.
37. Barbezat, G. O., and M. I. Grossman. 1971. Glucagon stimulates intestinal secretion. *Lancet.* **1**: 918.
38. Barbezat, G. O., and M. I. Grossman. 1971. Cholera-like diarrhea induced by glucagon plus gastrin. *Lancet.* **1**: 1025-1026.
39. Field, M. 1971. Ion transport in rabbit ileal mucosa. II. Effects of cyclic 3',5'-AMP. *Am. J. Physiol.* **221**: 992-997.
40. Dietz, J., and M. Field. 1973. Ion transport in rabbit ileal mucosa. IV. Bicarbonate secretion. *Am. J. Physiol.* **225**: 858-861.
41. Gardner, J. D., G. W. Peskin, J. J. Cerda, and F. P. Brooks. 1967. Alterations of *in vitro* fluid and electrolyte absorption by gastrointestinal hormones. *Am. J. Surg.* **113**: 57-64.
42. Sanzenbacher, L. J., H. S. Mekhjian, D. R. King, and R. M. Zollinger. 1972. Studies of the potential role of secretin in the islet cell tumor diarrheogenic syndrome. *Ann. Surg.* **176**: 394-402.
43. Hicks, T., and L. A. Turnberg. 1973. The influence of secretin on ion transport in the human jejunum. *Gut.* **14**: 485-490.
44. Gingell, J. C., M. W. Davies, and R. Shields. 1968. Effect of a synthetic gastrin-like pentapeptide upon the intestinal transport of sodium, potassium, and water. *Gut.* **9**: 111-116.
45. Bynum, T. E., E. D. Jacobson, and L. R. Johnson. 1971. Gastrin inhibition of intestinal absorption in dogs. *Gastroenterology.* **61**: 858-862.
46. Bussjaeger, L. J., and L. R. Johnson. 1973. Evidence for hormonal regulation of intestinal absorption by cholecystokinin. *Am. J. Physiol.* **224**: 1276-1279.
47. Gray, T. K., F. A. Bieberdorf, and J. S. Fordtran. 1973. Thyrocalcitonin and the jejunal absorption of calcium, water and electrolytes in normal subjects. *J. Clin. Invest.* **52**: 3084-3088.
48. Soergel, K. H., G. E. Whalen, J. A. Harris, and J. E. Greenen. 1968. Effect of antidiuretic hormone on human small intestinal water and solute transport. *J. Clin. Invest.* **47**: 1071-1082.
49. Hardcastle, P. T., and J. Eggenton. 1973. The effect of acetylcholine on the electrical activity of intestinal epithelial cells. *Biochim. Biophys. Acta.* **298**: 95-100.
50. Wright, R. D., M. A. Jennings, H. W. Florey, and R. Lium. 1940. The influence of nerves and drugs on secretion by the small intestine and investigation of the enzymes in intestinal juice. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **30**: 73-120.
51. Tidball, C. S. 1961. Active chloride transport during intestinal secretion. *Am. J. Physiol.* **200**: 309-312.
52. Brown, J. C., and J. R. Dryburgh. 1971. A gastric inhibitory polypeptide II: the complete amino acid sequence. *Can. J. Biochem.* **9**: 867-872.
53. Brown, J. C., V. Mutt, and R. A. Pederson. 1970. Further purification of a polypeptide demonstrating enterogastrone activity. *J. Physiol. (Lond.).* **209**: 57-64.
54. Pederson, R. A., and J. C. Brown. 1972. Inhibition of histamine-, pentagastrin-, and insulin-stimulated canine gastric secretion by pure "gastric inhibitory polypeptide". *Gastroenterology.* **62**: 393-400.
55. Johnson, L. R., and M. I. Grossman. 1968. Secretin: the enterogastrone released by acid in duodenum. *Am. J. Physiol.* **215**: 885-888.
56. Lin, T. M., and G. F. Spray. 1968. Effect of glucagon on gastric HCl secretion. *Gastroenterology.* **54**: 1254 (Abstr.)
57. Makman, M. H., and E. W. Sutherland, Jr. 1964. Use of liver adenylyl cyclase for assay of glucagon in human gastro-intestinal tract and pancreas. *Endocrinology.* **75**: 127-134.
58. Pohl, S. L., L. Birnbaumer, and M. Rodbell. 1969. Glucagon-sensitive adenylyl cyclase in plasma membrane of hepatic parenchyma cells. *Science (Wash. D. C.).* **164**: 566-567.
59. Pohl, S. L., L. Birnbaumer, and M. Rodbell. 1971. The glucagon-sensitive adenylyl cyclase system in plasma membranes of rat liver. I. Properties. *J. Biol. Chem.* **246**: 1849-1856.
60. Birnbaumer, L., and M. Rodbell. 1969. Adenylyl cyclase in fat cells. II. Hormone receptors. *J. Biol. Chem.* **244**: 3477-3482.
61. Bär, H. P., and O. Hechter. 1969. Adenylyl cyclase and hormone action. I. Effects of adrenocorticotrophic hormone, glucagon, and epinephrine on the plasma membrane of rat fat cells. *Proc. Natl. Acad. Sci. U. S. A.* **63**: 350-356.
62. Rodbell, M., L. Birnbaumer, and S. L. Pohl. 1970. Adenylyl cyclase in fat cells. III. Stimulation by secretin and the effects of trypsin on the receptors for lipolytic hormones. *J. Biol. Chem.* **245**: 718-722.
63. Murad, F., and M. Vaughan. 1969. Effect of glucagon on rat heart adenylyl cyclase. *Biochem. Pharmacol.* **18**: 1053-1059.
64. Levey, G. S., and S. E. Epstein. 1969. Activation of adenylyl cyclase by glucagon in cat and human heart. *Circ. Res.* **24**: 151-156.
65. Rutten, W. J., J. J. H. M. De Pont, and S. L. Bonting. 1972. Adenylyl cyclase in the rat pancreas, properties and stimulation by hormones. *Biochim. Biophys. Acta.* **274**: 201-213.
66. Marois, C., J. Morisset, and J. Dunnigan. 1972. Presence and stimulation of adenylyl cyclase in pancreas homogenate. *Rev. Can. Biol.* **31**: 253-257.
67. Bataille, D. P., P. Freychet, P. E. Kitabgi, and G. E. Rosselin. 1973. Gut glucagon: a common receptor site with pancreatic glucagon in liver cell plasma membranes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **30**: 215-218.