Effect of Vasoactive Intestinal Polypeptide (VIP) on the Net Movement of Electrolytes and Water and Glucose Absorption in the Jejunal Loop of Sheep

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Abstract. The effect of vasoactive intestinal polypeptide (VIP) on glucose absorption and the net movement of electrolytes and water in the jejunal loop of sheep was investigated using a Thiry-Vella loop. Intraluminal perfusion of glucose solution (10 mM) containing NaCl (149 mM) and PEG (1 mg/ml) was done at 1 ml/min and the outflow solution was collected every ten minutes. After a 30 min control period, VIP was infused into the jejunal loop at a rate of 10, 30, 100, 300, and 1,000 pmol/kg/hr. In the control period, water, sodium, chloride and glucose were absorbed, while bicarbonate and potassium were secreted. VIP decreased water absorption at 10 and 30 pmol/kg/hr and converted to secretion at over 100 pmol/kg/hr in a dose-dependent manner. Sodium flux changed to secretion only at 1,000 pmol/kg/hr, but chloride flux remained absorptive even at the highest dose. Bicarbonate secretion was stimulated dose-dependently by VIP. Potassium secretion was also increased at all doses, though this response was not dose-dependent. The net glucose absorption was not altered by VIP at any dose. Our findings indicate that VIP stimulates the jejunal secretion of water, sodium, potassium and bicarbonate and that VIP does not inhibit glucose absorption when the secretion of luminal fluid is accelerated by VIP in the jejunal loop of sheep. — Key words: electrolyte secretion, glucose absorption, intestinal loop, sheep, VIP.


Vasoactive intestinal polypeptide (VIP) is widely distributed throughout the body, and the highest concentration of VIP has been demonstrated in the submucosa of the rat [2] and the hamster intestine [3]. In the small intestine, VIP was shown to accelerate water and electrolyte secretion in the crypts of both humans [13] and monogastric animals [3, 4, 7, 12, 16], and to inhibit sodium, chloride and sugar absorption in the villous cells of the rabbit [1, 22]. The action of VIP is probably mediated by the increase of intracellular cAMP in the intestinal mucosa [5, 17, 20, 23, 26, 27]. Although most interest on the effect of VIP in the intestine has been paid to monogastric animals, the effect of this secretagogue on ruminants has not been reported yet. Thus, the present study was designed to evaluate the physiological and pathological effects of VIP on the net movement of water and electrolytes, and on glucose absorption in the small intestine of sheep. In the present study, the dose of VIP which brought a pathological rise of plasma VIP in humans, because the available data on pathological change with the watery diarrhea associated by VIP was not known in ruminants.

Materials and Methods

Animals and diets: Five male sheep weighing 42 to 48 kg were used. For acclimatization, they were kept in individual crates and fed orchard grass hay (100 g) and lucerne pellets (1,000 g) once daily at 19:00 to unify the experimental conditions by feeding [18]. Feed was generally consume within 2 hr of presentation. The animals were allowed free access to water.

Surgical preparation: The animals were fasted for 36 hr before surgery and atropine 0.066 mg/kg (atropine sulfate; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and xylazine 0.4 mg/kg (Celtacal; Bayer, Germany) were injected intramuscularly as a premedication. The sheep were anaesthetized with halothane (Fluothane; Takeda Pharmaceutical Industries Ltd., Osaka, Japan) during the laparotomy on the right flank as previously described [10]. A jejunal segment approximately 20 cm in length was isolated. Special care was paid to maintain mesenteric circulation and innervation intact. Both ends of the intestinal segment were connected with silicone catheters (ID 4 mm, OD 7 mm) by pursestring suture and the catheters were exteriorized through the right lateral abdominal wall. The continuity of the remaining intestinal tract was restored by end-to-end anastomosis and returned to the abdominal cavity.

Intestinal perfusion: Luminal perfusion was started from a week after the surgery. On the day of the experiment, a polyethylene catheter for VIP infusion was inserted into the right jugular vein at least 2 hr before experiments began. The stock solution (10 nmol/ml) of VIP (Peptide Institute, Inc., Osaka, Japan) was made with sterile 0.9% (w/v) NaCl containing 0.2% bovine serum albumin (A-7888, fraction V, RIA grade, Sigma, U.S.A.), and then diluted with sterile 0.9% (w/v) NaCl solution to the appropriate concentrations immediately before infusion.

The perfusate was composed of 149 mM NaCl and 10 mM glucose, and polyethylene glycol (PEG) was added to the perfusate at a concentration of 1 mg/ml as a non-absorbable marker. Perfusion was pumped into the loop at a flow rate of 1 ml/min for 150 min by a peristaltic pump (SJ-1211H, ATTO Co., Ltd., Tokyo, Japan) through a warm water-jacket at 38°C prior to entering the jejunal segment. The glucose concentration and perfusion rate used in this study were determined according to the previous report [10].
After washing the inside of the loop for the first 30 min of the 150 min perfusion period, the samples were collected for 120 min at every 10 min interval. After a second 30 min period of control, VIP was infused into the right jugular vein for 30 min by a peristaltic pump (SJ-1211H, ATTO Co., Ltd., Tokyo, Japan) at a dose of 10, 30, 100, 300 and 1,000 pmol/kg/hr. As a control, isotonic saline solution was infused for 30 min. In each animal, the experiment on control and each VIP dose were performed at least 1-day intervals within one month after the surgery. The order of experiments was randomized and limited to one-experiment a day. During the intervals, the jejunal loop was rinsed once a day with saline in order to clean the excessive mucus.

Analyses: The samples collected at 10 min intervals were immediately used for analysis after each experiment. Mean values of three 10 min measurements during the control period and infusion period were used for comparison. The amount of glucose which disappeared during the passing through the loop was expressed as the net absorption of glucose. The fluxes of electrolytes were calculated as the difference between the input and the output. Net movement of water was calculated by the concentration change of PEG in the outflow solution. Glucose concentration was determined by the glucose oxidase method [11]. Sodium and potassium concentrations were determined by flame spectrophotometer, bicarbonate by alkali-acid titration and chloride by Schales and Schales method [25]. Polyethylene glycol concentration was measured according to the method of Hydén [9].

Statistical analysis: All results were expressed as a mean ± SEM of net fluxes. The positive and negative values indicates net secretion and absorption respectively. Student’s t-test for paired or unpaired values was used for statistical analysis. A probability of less than 5% was considered statistically significant.

RESULTS

Figure 1 shows the time course of changes (A) and the dose-response relationships (B) in net movement of water after saline (control) or VIP infusion. In the control experiments, saline did not affect the net movement of water (\(-84.2 ± 19.1 \mu l/10 \text{ min/loop}\)) compared to the preinfusion period (\(-79.6 ± 15.9 \mu l/10 \text{ min/loop}\)) (Fig. 1A). At 10 and 30 pmol/kg/hr, VIP slightly decreased the water absorption compared to saline infusion (control), and the threshold dose of VIP infusion for reversion from net absorption to net secretion of water was 30–100 pmol/kg/hr (Fig. 1B). These significant secretory responses were observed at 10 min after the beginning of VIP infusion at 300 and 1,000 pmol/kg/hr, and at 20 min at 100 pmol/kg/hr. The maximal responses were observed at the end of VIP infusion, and this secretory effect ceased within 10 minutes after the end of VIP infusion (Fig. 1A).

Figure 2 shows the dose-response relationships of the net movement of sodium and chloride during the VIP infusion at doses of 10 to 1,000 pmol/kg/hr. The net movement of sodium was not changed by the infusion of VIP from 10 to 300 pmol/kg/hr, but it was reversed to secretion by the infusion at 1,000 pmol/kg/hr.

Figure 3 shows the dose-response relationships of the net secretion of potassium and bicarbonate during the VIP infusion at doses of 10 to 1,000 pmol/kg/hr. VIP significantly increased the secretion of bicarbonate in a dose-dependent manner. The threshold dose of VIP infusion for response of bicarbonate secretion was 10–30 pmol/kg/hr. The net secretion of potassium was significantly higher (19.1 ± 0.8 μEq/10 min/loop) at the lowest dose of VIP than that of the control (14.9 ± 1.0 μEq/10 min/loop). This response, however, was not dose-dependent.

![Figure 1](image1.png)

Fig. 1. The changes in the net movement of water after intravenous infusion of saline or VIP (A) and the relationship between VIP concentration and the net movement of water (B) in the jejunal loop of sheep. Net absorption from the lumen was expressed as a negative and net secretion as a positive value. The horizontal line represents the infusion period (30 min) of saline (control) or VIP. Values are shown by mean ± SEM of five sheep. Open symbols (A) indicate significant differences (p<0.05) from the pre-infusion value (mean of 30 min). Asterisk (B) shows significant difference from control, *p<0.05; **p<0.01.
**DISCUSSION**

The range of VIP doses used in this study was comparatively lower than those in vivo studies in nonruminants in which doses from 0.1 to 100 nmol/kg/hr were used [3, 12, 13, 22]. However, in the range used in the present study, Zabieliski et al. [28] demonstrated that VIP infused intravenously significantly stimulated pancreatic juice flow, bicarbonate and protein output in preruminating calves. In the present study, VIP at 100 pmol/kg/hr significantly stimulated the secretion of water, potassium and bicarbonate, while the movements of sodium and chloride remained completely unaffected. At the highest dose, water and bicarbonate secretions were markedly increased, and the net movement of sodium was reversed from absorption to secretion, but the chloride movement was still unaffected. These data suggest that the higher doses were effective to stimulate intestinal fluid and bicarbonate secretions in the jejunal loop of sheep, though chloride movement was not changed by these doses. This effect of VIP on intestinal secretion agrees with the in vivo studies in nonruminants [3, 13]. This secretory effect has been reported to cease shortly after the end of VIP infusion [14, 16]. Also in the present study, the response to VIP was restored to the basal level soon after stopping infusion.
It has been reported that plasma VIP levels in humans rose two- to four-fold by VIP infusion at 100 pmol/kg/hr, and rose to levels commonly observed in patients with pancreatic cholera syndrome by 200 and 400 pmol/kg/hr VIP infusion [13]. In anesthetized lambs, Reid et al. [21] observed that the basal VIP concentration in both gastric and intestinal venous plasma was undetectable or very low, and both were increased by electrical stimulation of the vagal nerve. However, the pathological change of plasma VIP level and the relationship between the infusion rate of VIP and its plasma level were not known in ruminants. In the present study, we employed the dose of VIP which brought a pathological rise of plasma VIP in humans. The dose of VIP used in this study was effective to induce the secretory responses of water, sodium, potassium and bicarbonate.

In most instances, VIP acts as a local neurotransmitter or neuromodulator [23, 24]. However, massively released VIP level by pathological conditions such as VIP-secreting tumors may be elevated in the general circulation, and the peptide under those conditions can act as a circulating hormone on remote targets [23]. In epithelial cells of the intestine, the biologic effects of VIP seem to be largely mediated by its binding to specific cell-surface receptors, which results in the increased production of intracellular cAMP via adenylyl cyclase stimulation, because VIP binding and VIP-induced cAMP production were observed in a similar pattern in isolated rat intestinal epithelial cells [19] and specific binding of 125I-labeled VIP was demonstrated only on the basolateral membrane of enterocytes of the rabbit ileum and rat jejunum [6].

Anionic secretion caused by VIP differs according to the species and intestinal segments. In the duodenum, VIP induced chloride and bicarbonate secretion in rats [3]. In the jejunum, VIP induced active chloride and bicarbonate secretions in dogs [12], whereas only a chloride secretion was induced in man [5, 13] and in rats [3] via the activation of the apical chloride channel by cAMP-dependent protein kinases [6, 15, 26]. In the ileum, bicarbonate secretion was associated with the suppression of chloride absorption in rats [3], while only chloride was secreted in rabbits [26]. In the present study, VIP stimulated bicarbonate secretion without changing chloride flux. It suggests that VIP stimulates the secretion of bicarbonate rather than chloride as an anion in the ovine jejunum, and this bicarbonate secretion induced by VIP occurs via a chloride-independent process. The minimal effective dose of VIP to stimulate bicarbonate secretion was 30 pmol/kg/hr. However, the dose to obtain the maximal response was not determined in this study because the secretory response of bicarbonate did not reach to the plateau level in the range of VIP dose used in this study. McCabe and Dharmasathaphorn [17] observed that the stimulation of the potassium efflux pathway by VIP was concurrent with the activation of electrogenic chloride secretion. However, our data provided no evidence in support of such an effect.

Although most interest in VIP has been paid to the secretory effect, little is known about the effect of VIP on sugar transport in the small intestine. Coupar [4] and Davis et al. [5] demonstrated that glucose absorption across the brush border and lateral membranes was not affected during VIP infusion both in rats [4] and in man [5], but galactose absorption was inhibited in the rabbit jejunum [1, 22]. In the present study, the effective doses to stimulate intestinal secretion did not alter glucose absorption. Furthermore, the maximal dose of VIP also did not affect glucose absorption when the glucose concentration in the perfusate was varied in a range from 5 to 40 mM (our unpublished observation).

It has been reported that VIP was scarcely distributed in the apex of the villi, while it was plentifully distributed in the crypt area in rats [2] and in hamsters [8]. This evidence suggests that VIP may play a primary role in the secretory cells rather than in the absorptive cells. However, this morphological evidence on the distribution of VIP has not yet been studied in ruminants. Thus, further investigations are needed to clarify the role of VIP in the villous cells of ruminants.

In conclusion, we demonstrated that the absorptive mechanisms for glucose in the villous cells were not altered when the secretion of water and bicarbonate were accelerated by intravenous infusion of VIP in the ovine small intestine. However, a comparative study using other secretagogues such as PGE_{2} will help to clarify the relationship between absorptive and secretory function in the small intestine of ruminants.

REFERENCES

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