Substance P-induced intestinal secretion of water and electrolytes

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SUMMARY This study was initiated to determine if raised (carcinoid) plasma concentrations of substance P induced jejunal secretion of water and electrolytes. Five dogs had isolated and cannulated 25 cm jejunal segments perfused at 2 ml/min with a neutral, isotonic perfusate. Saline, 1.0 ml, was infused intravenously during basal and recovery periods, while substance P was administered intravenously at 75 ng/kg/min (55 pmol/kg/min) during the four 15 minute experimental periods. Infusion increased plasma SP concentrations from basal (5.8 ± 1.3 pg/ml) to a mean plateau level of 121.2 ± 25.2 pg/ml (mean $\pm$ SEM). During SP infusion, intestinal secretion of water, Na^+ , and Cl^- were documented (H_2O basal $+102 \pm 60$ to SP -275 ± 60 ; $\mu\text{l}/\text{min}$; Na^+ basal $+19.8 \pm 7.2$ to SP -23.2 ± 7.5 $\mu\text{Eq}/\text{min}$; Cl^- basal 21.7 ± 7.5 to SP -16.5 ± 5.6 $\mu\text{Eq}/\text{min}$). Under basal conditions, there was minimal secretion of potassium (-0.264 ± 0.282 $\mu\text{Eq}/\text{min}$); during SP infusion, K^+ flux was altered to significant secretion (-1.784 ± 0.271 $\mu\text{Eq}/\text{min}$). Serum concentrations of Na and Cl were unchanged during SP infusion, but serum potassium concentrations fell from 4.64 ± 0.12 to 3.85 ± 0.40 mEq/l. The data demonstrate that substance P at levels noted in the carcinoid syndrome induces significant jejunal secretion of water and electrolytes in the dog.

In 1931 von Euler and Gaddum described a hypotensive and spasmogenic extract from horse brain and intestine and named it substance P.¹ This extract was later shown to be an 11 amino acid oligopeptide extensively distributed in both the nervous and gastrointestinal systems. Central nervous system substance P is predominantly localised within the grey matter, the dorsal half of the spinal cord, and the primary sensory neurones of the spinal ganglia.^{2,3} In the gastrointestinal tract SP is diffusely distributed, with the highest concentrations in the small intestine.^{4,5} Intramural intestinal substance P has been localised in the mucosal enterochromaffin cells⁶ and most components of the submucosal nervous plexus.²

Various systemic actions have been attributed to substance P including alterations in the distribution of blood flow,^{7,8} glucose homeostasis,⁹ and water balance.¹⁰ Gastrointestinal manifestations of the peptide include profound increases in motility,^{2,11} alterations in exocrine biliary and pancreatic secre-

tion,^{12,13} and increases in blood flow to both ileal and fundic muscularis.⁷ A role in the intestinal modulation of water and electrolyte management has also been suggested by *in vitro* studies.^{14,15}

Immunohistochemical and immunoassay studies have identified high concentrations of substance P in carcinoid tumours¹⁶ as well as in the blood of patients with the carcinoid syndrome.^{17,18} The present experiments were undertaken to determine whether raised plasma levels of substance P, simulating those reported in the carcinoid syndrome, were capable of inducing alterations in the intestinal management of water and electrolyte balance in an awake canine model. These observations have allowed us to speculate as to the role of circulating substance P as a potential mediator of the carcinoid syndrome.

Methods

DOGS

Under general anaesthesia, five 18-25 kg mongrel dogs had 25 cm proximal jejunal segments isolated and cannulated at each end. The cannulae exited through lateral abdominal stab wounds and were

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Received for publication 17 June 1985.

sutured in place. The dogs were placed on systemic antibiotics for 48 hours perioperatively and allowed to recover for at least two weeks before experimentation. During the course of the study, the dogs remained healthy and maintained or gained weight on a standard *ad libitum* diet.

Experiments were carried out once weekly on each dog after an overnight fast but allowing free access to water. In each study, the jejunal segment was perfused through the proximal cannula at 2 ml/min via a roller pump (Sage Instruments, Cambridge, MA) and the perfusates were volumetrically collected from the distal cannula for water and electrolyte measurements. A water bath was used to maintain the perfusate temperature at 37°C. The perfusate,¹⁸ buffered to a pH of 7.4, contained 140 mmol Na⁺, 5.2 mmol K⁺, 119.8 mmol Cl⁻, 25 mmol HCO₃⁻, 1.2 mmol Ca⁺⁺, 1.2 mmol Mg⁺⁺, 2.4 mmol HPO₄⁼, 0.4 mmol H₂PO₄⁼, and 10 µCi ¹⁴C-polyethylene glycol (¹⁴C-PEG) in 5 g/l of carrier PEG as a volume marker. Because of cross-ionic bonding, mannitol was added to the solution to assure a final osmolality of 280–290 mOsm/l. The segment was irrigated free of mucous during a 15 min luminal washout period. During the initial two 15 min control periods, normal saline was infused in a forelimb vein (Harvard Apparatus, Miller, MA) at 1.0 ml/min while the jejunal segment was perfused with the specific perfusate. For the four subsequent 15 min study periods, pure synthetic porcine substance P (Peninsula Laboratories, San Carlos, CA) was infused intravenously at 75 ng/kg/min (55 pmol/kg/min) in normal saline (containing 1 ml autologous plasma as the carrier protein) adjusted to a rate of 1.0 ml/min. After the experimental periods of substance P infusion, the intravenous infusions were changed back to saline and two 15 min recovery periods of jejunal perfusion completed each study. Perfusate aliquots collected from the distal cannula during each 15 min period were stored frozen at 20°C until ¹⁴C-PEG, water, and electrolyte measurements were completed. Using a separate hindlimb venous catheter, venous blood was collected every 15 min during the study in EDTA tubes containing 500 U/ml Aprotinin for measurements of plasma substance P concentrations. Additional hindlimb venous blood samples were collected in glass tubes for serum electrolyte measurements.

Sodium and potassium ion concentrations were determined by flame photometry (Instrumentation Laboratory, Lexington, MA). Chloride ion concentrations were measured by a Buchler-Cotlove chloridometer (Buchler Instruments, Fort Lee, NJ). Osmolality was determined by freezing point depression (Advanced Instruments, Needham

Heights, MA). ¹⁴C-PEG activity was assayed by liquid scintillation scanning (Packard Instruments, Downers Grove, IL) after dissolution in 5 ml Aquasure™ scintillation fluid (New England Nuclear, Boston, MA).

For each 15 min period, net water flux in the segment (expressed as µl/min) was calculated from the change in ¹⁴C-PEG concentration and the perfusion rate (2 ml/min) using the formula:

$$F_{H_2O} = \text{perfusion rate} \times 1 - \frac{(^{14}\text{C-PEG}) \text{ infusate}}{(^{14}\text{C-PEG}) \text{ effluent}}$$

Net ion flux was expressed in µEq/min and calculated from:

$$F_{\text{ion}} = \text{Perfusion Rate} \times (\text{Ion})_{\text{infusate}} - (\text{Ion})_{\text{effluent}} \times \frac{(^{14}\text{C-PEG}) \text{ infusate}}{(^{14}\text{C-PEG}) \text{ effluent}}$$

Recovery of ¹⁴C-PEG (R) was calculated by the formula¹⁹:

$$R = \frac{(^{14}\text{C-PEG}) \text{ effluent} \times \text{volume out} \times 100}{(^{14}\text{C-PEG}) \text{ infusate} \times \text{volume in}}$$

Recoveries not within 100% ± 5% were rejected and data from those study periods discarded.

Blood samples were collected on ice and, within 60 minutes of the cessation of each experiment, were centrifuged at 3000 rpm × 30 minutes at 4°C to obtain plasma specimens, which were stored at -20°C until assay. Substance P concentrations were determined on extracted plasma by a radioimmunoassay developed in our laboratory.²⁰ Serum samples were stored at -20°C until electrolyte measurements were done.

Statistical analyses were done using the Student's *t* test for unpaired data, with significance accepted at the 5% level. Results are expressed as mean ± SEM.

Results

Basal extracted venous plasma substance P concentrations averaged 5.8 ± 1.3 pg/ml. Substance P concentrations reached a plateau by 30 minutes into the substance P infusion and averaged 121.2 ± 25.2 pg/ml for the entire period of infusion. These values correlate with published ranges of normal (<10 pg/ml) and carcinoid levels (80–200 pg/ml) of peripheral blood substance P in man.⁸ Substance P concentrations rapidly declined after cessation of the infusions, averaging 6.0 ± 1.7 pg/ml for the recovery periods.

Neither serum sodium nor chloride concentrations changed significantly from control values (142.2 ± 1.6 and 108.8 ± 1.5 mmol/l, respectively) during or after the substance P infusions. In contrast, serum potassium concentrations were significantly depressed during the substance P infusions (4.64 ± 0.12 mmol/l vs 4.32 ± 0.16 mmol/l, $p < 0.05$) and remained low during the recovery periods (3.85 ± 0.40 mmol/l, $p < 0.05$).

Jejunal fluxes of water and electrolytes (f_{H_2O} and

f_{ion}) are presented in $\mu\text{l}/\text{min}$ and $\mu\text{Eq}/\text{min}$, respectively. Positive numbers denote net absorption, negative values, net secretion. In the basal state, the expected net absorption of water, sodium, and chloride was noted, with the more passive potassium flux minimally favoring secretion. Within 15 minutes of the onset of the substance P infusions, statistically significant net secretion of water, sodium, chloride, and potassium was observed ($p < 0.05$). Secretion continued throughout the entire 60 minute sub-

Table Fluxes of water and electrolytes

	H_2O ($\mu\text{l}/\text{min}$)	Na^+ ($\mu\text{Eq}/\text{min}$)	Cl^- ($\mu\text{Eq}/\text{min}$)	K^+ ($\mu\text{Eq}/\text{min}$)
iv saline (basal)	102 ± 60	19.8 ± 7.2	21.7 ± 7.5	-0.264 ± 0.282
iv SP				
0-15 min	$-43 \pm 105^*$	$-1.9 \pm 10.3^*$	$+4.9 \pm 9.9^*$	$-1.170 \pm 0.055^*$
15-30 min	$-375 \pm 199^*$	$-12.1 \pm 11.1^*$	$-11.7 \pm 9.2^*$	-1.066 ± 0.352
30-45 min	$-374 \pm 59^*$	$-39.4 \pm 11.6^*$	$-35.1 \pm 10.9^*$	$-2.505 \pm 0.692^*$
45-60 min	$-308 \pm 56^*$	$-39.3 \pm 22.2^*$	$-24.1 \pm 12.1^*$	$-1.928 \pm 0.385^*$
iv saline (recovery)				
0-15 min	$-226 \pm 115^*$	$-19.9 \pm 14.8^*$	$-3.9 \pm 12.8^*$	$-1.585 \pm 0.417^*$
15-30 min	$+72 \pm 63$	$+19.3 \pm 11.7$	$+10.5 \pm 10.8$	-0.005 ± 0.426

* $p < 0.01$ compared with basal

Figure Effect of substance P infusion on jejunal fluxes of (a) water (b) Na^+ and Cl^- , and (c) K^+ .

stance P infusion periods without significant fluctuation (Table). Fifteen minutes after termination of the infusions, ion and water fluxes began to return toward control values, and they reached pre-infusion ranges by 30 minutes postinfusion.

Overall summed data for the basal, infusion, and recovery periods are presented in the Figure. The data show significant secretory effects on the jejunal mucosa induced by raised plasma substance P concentrations. Water fluxes changed from net $+102 \pm 60$ $\mu\text{l}/\text{min}$ (absorption) to -275 ± 60 $\mu\text{l}/\text{min}$ (secretion) during substance P infusion ($p < 0.001$) then recovered to $+72 \pm 63$ $\mu\text{l}/\text{min}$ ($p < 0.001$) sodium fluxes changed from $+19.8 \pm 7.2$ $\mu\text{Eq}/\text{min}$ basally to -23.2 ± 7.5 $\mu\text{Eq}/\text{min}$ during substance P infusion ($p < 0.001$) and reversed to $+19.3 \pm 11.7$ $\mu\text{Eq}/\text{min}$ during recovery ($p < 0.005$). Chloride fluxes paralleled sodium fluxes, averaging $+21.7 \pm 7.5$ $\mu\text{Eq}/\text{min}$ during basal periods, -16.5 ± 5.6 $\mu\text{Eq}/\text{min}$ during the substance P infusions ($p < 0.001$), and $+10.5 \pm 10.8$ $\mu\text{Eq}/\text{min}$ during the recovery periods ($p < 0.02$). Net potassium fluxes, minimally secretory during the basal periods at -0.264 ± 0.282 $\mu\text{Eq}/\text{min}$, changed to -1.784 ± 0.271 $\mu\text{Eq}/\text{min}$ during substance P infusion ($p < 0.001$), and returned to -0.005 ± 0.426 $\mu\text{Eq}/\text{min}$ during recovery ($p < 0.001$).

Discussion

In these experiments we have attempted to elucidate the effects on jejunal secretion and absorption of duplicating the supranormal levels of substance P seen in the carcinoid syndrome. Data from our laboratory⁸ and others¹⁷⁻²¹ have revealed significantly raised circulating plasma substance P concentrations in patients with the carcinoid syndrome, ranging from 82–220 pg/ml. Classically, the hyper-serotoninaemia seen in many carcinoid syndrome patients is thought to induce profuse diarrhoea frequently seen in this disorder, and both animal²² and man²³ studies have shown serotonin to be a potent intestinal secretagogue *in vivo*. The failure of some carcinoid patients to respond to antiserotonin antidiarrhoeal agents²³ has led to speculation on the role of other circulating secretagogues, including substance P.

Substance P is well documented as a stimulator of intestinal motility in mammals²; it is more potent than either acetylcholine or histamine in this regard.²⁴⁻²⁵ The early observations of large concentrations of substance P in the intestinal wall and of its impressive spasmogenic properties has suggested that it might be an important modulator of intestinal motility.² Recently documented postprandial increases in plasma substance P concentrations²⁰ and the effects of intraluminal administration

on stimulation of mucosal blood flow²⁶ have encouraged its consideration as a multifactorial modulator of gastrointestinal tract homeostasis. Substance P is known to be a potent stimulator of salivary gland and pancreatic secretion in many species² apparently due to a direct, non-neural, effect on glandular cells.²⁷ The concentration of substance P fibres in the submucosa and mucosa of the small intestine, especially those in contiguity with secretory glands and villi, suggests a role in gut secretory and absorptive functions.² This study confirms the secretory effects of raised plasma substance P concentrations on the proximal jejunum of the awake dog. Possible physiologic mechanisms include a direct local effect on mucosal cells. This mechanism is supported by studies using large doses of substance P, which induced transient increases in transepithelial potential differences and short circuit currents in the serosal surfaces of both guinea pig and rat ileum.¹⁴⁻¹⁵ In addition, jejunal glucose absorption has also been shown to be decreased by substance P *in vivo*.²⁸ Other, as yet untested mechanisms of action include the central and/or peripheral neurologic effects of substance P as well as its interactions with other gastrointestinal peptides. Humoural mediation is suggested by the reports of a suppressive effect of substance P on gastric somatostatin release²⁹⁻³⁰ and of inhibition of carcinoid diarrhoea by somatostatin acting at the level of the small intestine.³¹

Along with other neurohumoural substances that alter intestinal transport, such as serotonin and neurotensin, substance P appears to effect intestinal transport by increasing Ca^{++} entry across the basolateral membrane of intestinal epithelial cells.³²⁻³³ Its secretory effects are unaffected *in vitro* by atropine, indomethacin, diphenhydramine, and somatostatin, but can be reversed by removal of extracellular Ca^{++} ions or the addition of Verapamil, a calcium-channel blocker, to the serosal bathing solution.¹⁵ Most current data support substance P as a calcium dependent local modulator of intestinal ion transport³⁴ and mucosal blood flow.²⁶

This study has supported the role of raised plasma concentrations of substance P in inducing intestinal secretion. Our data showed significant secretion of water, Na^+ , Cl^- , and K^+ . Mucosal electrogenic secretion of Cl^- is a well described phenomenon and it is possible that this is the predominant effect of substance P, with Na^+ , K^+ , and H_2O merely following electrochemical and osmotic gradients. This model does not allow us to discriminate between these effects any further. All of the animals were restless with profuse salivation during the substance P infusions, and 30% developed profound diarrhoea. The significant depression of serum K^+

concentrations were possibly because of the substantial intraluminal secretion of potassium during the infusions. Substance P was, however, also reported to stimulate potassium release from rat parotid slices.³⁵ In conclusion, our findings document a significant influence on jejunal handling of water and electrolytes in an awake animal model and support a possible role of substance P in the diarrhoeogenesis of the carcinoid syndrome.

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