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Jejunal water and electrolyte secretion induced by L-arginine in man

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SUMMARY In this study a perfusion technique has been used to investigate jejunal secretion in response to the dibasic amino acid L-arginine. L-arginine at 5, 15, and 40 mmol/l in isotonic saline solutions induced net intestinal secretion of water and Na^+. The structurally similar dibasic amino acid L-lysine caused net absorption at 5 and 15 mmol/l, and only modest net secretion of water and Na^+ at 40 mmol/l, although aborption rates of the two amino acids were similar. D-arginine (15 mmol/l) was without effect on net water and Na^+ absorption. L-arginine 15 mmol/l inhibited glucose-stimulated water and Na^+ absorption when perfused in the same intestinal segment, but was without effect when perfused in separate jejunal or ileal segments. Parenteral chlorpromazine inhibited L-arginine induced jejunal water and Na^+ secretion. Jejunal secretion induced by L-arginine thus appears not to be due to passive osmotic water flow, nor to release of circulating secretagogues. Stimulation of a mucosal secretory process is most likely to be the mechanism.

The role of intraluminal factors in the control of normal intestinal water and electrolyte homeostasis is poorly understood. Actively transported sugars and most amino acids stimulate intestinal absorption of water and Na^+ ion. Hellier *et al.*,[1] however, showed that the dibasic amino acid L-arginine caused water and Na^+ secretion when perfused in the human jejunum. The mechanism of this secretion was not understood. We have therefore reinvestigated the phenomenon of intestinal secretion induced by L-arginine.

Experiments have been performed to determine the effect of L-arginine on net water and sodium transport in the human jejunum and to compare this with the effects of the structurally similar dibasic amino acids D-arginine and L-lysine. The possibility that L-arginine releases circulating secretagogues has been examined *in vivo*, and inhibition of L-arginine induced secretion by chlorpromazine—a known inhibitor of cholera toxin induced secretion[2]—has been demonstrated.

Methods

PERFUSION SOLUTIONS

All perfusion solutions were rendered iso-osmotic (290 mOsm/kg) by the addition of sodium chloride and contained the non-absorbable marker polyethylene glycol (PEG, mw 4000) at a concentration of 2·5 g/l labelled with 1 μCi of ^{14}C PEG/l. Although PEG itself has been shown to induce intestinal secretion during jejunal perfusion studies in man,[3] secretion was significant only at higher concentrations than those used in the present study. In addition, as all the solutions used in our study contained the same amount of PEG, comparisons between them will still be valid. The pH of the solution was adjusted to 7·0 by the addition of 1M NaOH of HCl as appropriate. The amino acids and glucose were obtained from standard commercial sources and were of the highest available purity. The amino acids contained no ninhydrin-positive contaminants on ion exchange chromatography.

INTESTINAL PERFUSION STUDIES

Normal adult volunteers aged 20 to 55 years were intubated with a double lumen perfusion tube incorporating a 30 cm perfusion segment and a proximal occlusive balloon.[4] The tube was positioned under fluoroscopy so that the infusion orifice was within the 10 cm segment of jejunum distal to the duodenojejunal flexure. Perfusion solutions were infused at 15 ml/min from bottles maintained at 37°C using a peristaltic pump. After a 30 minute equilibration period, three 10 minute

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samples of aspirate were collected and stored at −20°C until required for analysis.

The following experiments were performed:

Experiment 1

Water and solute transport was measured during jejunal perfusion with (a) L-arginine 5, 15, and 40 mmol/l in six subjects, (b) L-lysine 5, 15, and 40 mmol/l in six subjects, and (c) D-arginine 15 mmol/l in six subjects. The solutions were perfused in random order.

Experiment 2

To search for evidence of release of circulating secretagogues three series of experiments were performed:

a. Four subjects were intubated with a four lumen tube incorporating two adjacent perfusion segments.[5] The proximal 20 cm segment was separated from the distal 30 cm segment by an occlusive balloon. The tube was positioned in the upper jejunum so that the infusion orifice of the proximal segment was just distal to the duodenojejunal flexure. The distal segment was then perfused with 10 mmol/l glucose-saline at 15 ml/min for 180 minutes and absorption from this segment was measured continuously. During the middle 60 minutes (study period) 40 mmol/l L-arginine in isotonic saline solution was perfused at 15 ml/min in the proximal perfusion segment. The efficiency of the occluding balloon was tested by adding phenol red to the solution perfused in the proximal segment, checking the aspirates from the distal segment for evidence of contamination by phenol red.

b. In four subjects water and solute movement was measured continuously for 180 minutes in a 30 cm jejunal segment during perfusion with 10 mmol/l glucose saline at 15 ml/min. During the middle 60 minutes (study period) 40 mmol/l L-arginine was infused into the distal small intestine 180 cm from the teeth at a rate of 15 ml/min *via* a separate single lumen intestinal tube.

c. In three subjects water and solute movement from a 30 cm jejunal segment perfused at 15 ml/min with a solution containing both 10 mMol/l glucose and 40 mMol/l L-arginine was measured.

Experiment 3

In six subjects, 15 mmol/l L-arginine was perfused at 15 ml/min in a 30 cm jejunal segment and water and electrolyte movements were monitored continuously for 180 minutes. In another three subjects, chlorpromazine (1·4 mg/kg body weight) was given intramuscularly 30 minutes after the establishment of a steady state, and water and electrolyte movements were monitored for another 150 minutes.

Local Ethical Committee permission for these studies was obtained and all subjects gave fully informed consent to the procedures here described.

ANALYTICAL METHODS

Amino acid concentrations were measured using a Locarte amino acid analyser (Locarte Company, London). ^{14}C PEG was measured in an LKB 1210 Ultrobeta liquid scintillation counter,[6] sodium by flame photometry using an EEL 227 instrument, chloride using an EEL chloride meter, glucose by a standard autoanalyser neocuprine method, and osmolality by freezing point depression using an Advanced osmometer. Absorption rates were calculated using previously described formulae.[4] The statistical significance of differences in absorption

Table 1 *Water and solute movements during jejunal perfusion with L- and D-arginine and L-lysine*

Amino acid	*Concn. (mmol/l)*	*H_2O movement (ml/h/30 cm)*	*Na^+ movement (mmol/h/30 cm)*	*Cl^- movement (mmol/h/30 cm)*	*Amino acid absorption (mmol/h/30 cm)*
L-Lysine	5	(+) 66·8±5·2	(+) 11·6±1·7	(+) 10·8±2·4	1·4±0·22
		(P<0·002)	(P)<0·005)	(P<0·002)	(NS)
L-arginine	5	(−) 99·8±13·1	(−) 13·1±1·2	(−) 12·9±2·3	1·7±0·31
L-lysine	15	(+)43±19·1	(+) 9·0±2·1	(+) 8·4±1·2	2·8±0·26
		(P<0·002)	(P<0·002)	(P<0·002)	(NS)
L-arginine	15	(−) 113±17·1	(−) 14·1±1·8	(−) 13·2±2·4	2·1±0·25
		(P<0·005)	(P<0·002)	(P<0·002)	(P<0·002)
D-arginine	15	(+) 17·9±13·8	(+) 3·6±2·2	(+) 3·8±2·4	0·12±0·04
L-lysine	40	(−) 33·0±6·0	(−) 2·4±1·6	(−) 3·2±0·9	2·5±0·7
		(P<0·0005)	(P<0·002)	(P<0·005)	(NS)
L-arginine	40	(−) 151±12·5	(−) 14·9±2·1	(−) 12·8±1·6	1·8±0·8

(+): absorption. (−): secretion. Values are the mean of six studies ±SEM.

rates was evaluated by the paired and unpaired *t* tests where appropriate.[7]

Results

EXPERIMENT 1

a. Net secretion of water, sodium, and chloride ion into the jejunal lumen was seen during perfusion of L-arginine (Table 1) even at 5 mmol/l, the lowest concentration studied. There was a graded increase in secretion with increasing L-arginine concentration.

b. L-lysine, however, had very different effects on jejunal water and sodium movement (Table 1). Net water and sodium absorption occurred from 5 and 15 mmol/l L-lysine and moderate net secretion (at only one-third of the rate observed with 5 mmol/l L-arginine) was seen with 40 mmol/l L-lysine.

These differences in water and sodium movement occurred despite the fact that amino acid absorption rates from equal concentrations of L-arginine and L-lysine were identical (Table 1).

c. Whereas net secretion of water and electrolytes was seen with 15 mmol/l L-arginine. net absorption of water and sodium was seen during perfusion with 15 mMol/l D-arginine, despite the fact that D-arginine was absorbed at only 6% of the rate of L-arginine (Table 1). The differences between absorption rates of water, sodium, and chloride are highly significant ($P < 0.005$ or less, Table 1).

EXPERIMENT 2

The results of this experiment are summarised in Tables 2 and 3. Net absorption of water and sodium ion was seen during perfusion of 10 mmol/l glucose in the jejunum. This absorption was unaffected by simultaneous perfusion of either (a) a proximal occluded jejunal segment or (b) a distal intestinal segment with 40 mmol/l L-arginine (Table 2). In contrast, when a mixture of 40 mmol/l L-arginine and 10 mmol/l D-glucose was perfused in the same intestinal segment (c) there was marked inhibition of the net water and sodium ion absorption seen with glucose alone (Table 3). The effects of glucose and L-arginine on water and sodium absorption were additive within the limits of accuracy of the experiment. Neither glucose nor L-arginine had any effect upon the absorption of the other (Table 3).

EXPERIMENT 3

Jejunal perfusion of 15 mmol/l L-arginine in six control subjects produced water secretion of 100 ±1SD 20 ml/h per 30 cm which was unchanged during the entire 180 minutes of steady state perfusion. In three other subjects given chlorpromazine (1·4 mg/kg intramuscularly) there was a sustained and highly significant reversal of L-arginine induced water secretion (which was greater than 3 SD from the mean of water secretion rates in the control subjects) beginning between 60 and 90 minutes after

Table 2 *Solute and water movements during jejunal perfusion of 10 mmol/l D-glucose: effect of simultaneous perfusion of 40 mmol/l L-arginine in (A) jejunum and (B) distal small intestine in four normal subjects*

Transport	*A. Jejunum*			*B. Distal small intestine*		
	Control[1]	*Study period*	*Control*[2]	*Control*[1]	*Study period*	*Control*[2]
Water (ml/h/30 cm)	100 ±14·2	101·7±10·3	102·5±8·1	129 ±13·3	132 ±13·4	132 ±15
Na^+ (mmol/h/30 cm)	12·2± 2·4	13·5± 1·9	13·3±2·0	13·2± 2·5	14·2± 3·1	12·8± 2·5
Cl (mmol/h/30 cm)	11·4± 3	13·1± 2·1	12·9±1·4	14·1± 2·9	13·9± 3·0	13·8± 1·7
Glucose (mmol/h/30 cm)	8·1± 0·9	7·9± 0·5	8·3±0·4	8·3± 0·6	8·9± 0·5	8·4± 0·3

Table 3 *Solute and water movements during jejunal perfusion with L-arginine (40 mmol/l) D-glucose (10 mmol/l), and a mixture of D-glucose (10 mmol/l) and L-arginine (40 mmol/l) in six subjects*

Perfusion solution	*Transport*			*Absorption*	
	Water (ml/h/30 cm)	*Na^+ (mmol/h/30 cm)*	*Cl (mmol/h/30 cm)*	*Glucose (mmol/h/30 cm)*	*Arginine (mmol/h/30 cm)*
L-arginine (40 mmol/l)	(−) 129 ±18·1	(−) 12·9±2·1	(−) 13·7±3·0	—	2·1±0·7
Glucose (10 mmol/l)	(+) 113·5±10·2	(+) 13·3±2·8	(+) 14·1±1·7	8·0±0·6	—
	($P < 0.002$)	($P < 0.005$)	($P < 0.002$)	(NS)	(NS)
Glucose (10 mmol/l) L-arginine (40 mmol/l)	(+) 10·1±15·3	(+) 1·7 ±1·1	(+) 2·3±1·4	8·2±1·0	1·9±0·4

Results are mean of six studies ±SEM. (−): secretion. (+): absorption.

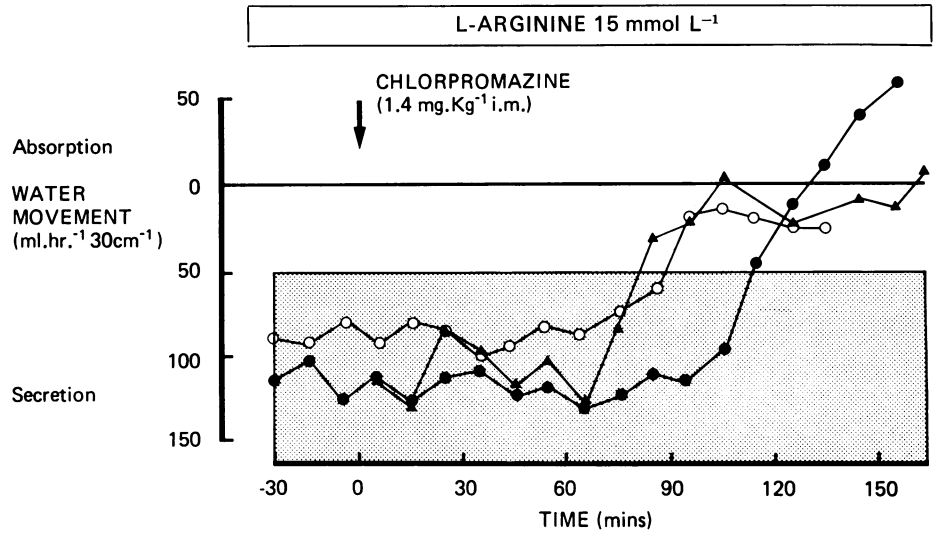

Fig. *Effect of chlorpromazine on jejunal water secretion induced by perfusion of 15 mmol/l L-arginine in isotonic saline solution. The hatched area is the normal range of L-arginine induced water secretion (mean ±3SD) in six normal subjects.* ○–○, ●–●, *and* ▲–▲ *indicate three other subjects given chlorpromazine 1·4 mg/kg intramuscularly at a time 0.*

administration of the drug, and persisting to the end of the experiment (Figure).

Discussion

Stimulation of intestinal electrolyte secretion by a number of amino acids and monosaccharides[8 9] has been demonstrated *in vitro,* but *in vivo* in man only L-arginine has been shown to have this effect.[1] Hellier *et al.*[1] attributed the secretion to a toxic effect of L-arginine on the mucosa but did not investigate the mechanism of the secretion.

Using our double lumen perfusion system in the human jejunum, there is normally no significant net water or Na^+ absorption from isotonic saline solutions.[10 11] Low concentrations of glucose,[10] amino acids,[1 11 12] peptides,[1 11] or bicarbonate ion[13 14] in the perfusate stimulate significant net water and Na^+ absorption. L-lysine at 5 and 15 mmol/l seems to share this property. In this study L-arginine, however, induced highly significant water and Na^+ secretion over the concentration range 5–40 mmol/l. We have no ready explanation for the fact that Hellier *et al.* were able to show secretion of water and Na^+ only at concentrations of L-arginine above 10 mmol/l. L-lysine at 40 mmol/l also causes secretion but is a much less powerful stimulus than L-arginine. These differences in the effect of L-lysine and L-arginine are surprising in view of the similarities in molecular structure, size, pK values, and absorption rates of the two amino acids. Because the absorption rates of L-lysine and L-arginine are similar, the residual concentration of osmotically active amino acid in the intestinal lumen is likewise similar, suggesting that the secretory effect of L-arginine is not due purely to the osmotic effect of unabsorbed solute. This impression is confirmed by the studies with D-arginine. D and L-arginine are stereoisomers and thus have identical physical-chemical characteristics. As expected, the naturally occurring L-isomer was absorbed much more rapidly than the D-isomer when both were perfused at 15 mmol/l. The residual intraluminal concentration of D-arginine was therefore greater than that of L-arginine and yet D-arginine did not cause secretion of water and Na^+ ion. These differences between the effects of D- and L-arginine might indicate a stereospecific effect, but could equally well be due to the presumably lower concentrations of the D-isomer within the epithelium. These observations, however, do seem to exclude the possiblity that L-arginine induces intestinal secretion by purely osmotic mechanisms.

The possibility that L-arginine could be producing secretion by releasing circulating secretagogues has been considered. Release of a variety of endogenous hormones after intravenous L-arginine is well documented.[15 16] Post-prandial rise of levels of a variety of gastrointestinal hormones has been demonstrated and some of these, including secretin,[17] glucagon,[18] and somatostatin,[19] when infused intravenously have been shown to modify the intestinal

transport of water and electrolytes, albeit at unphysiological plasma levels. Furthermore, intravenous administration of a mixture of gastrin, GIP, cholecystokinin, glucagon, and secretin producing plasma levels similar to those after a meal induces net secretion of water and Na^+ ion during jejunal perfusion of electrolyte solutions in man.[20] Release of circulating secretagogues from the intestine during L-arginine perfusion was evaluated using the double perfusion technique. A submaximal stimulation of water and Na^+ absorption was induced by 10 mmol/l D-glucose to render more obvious any subtle effects on water and Na^+ movement induced by circulating hormones. L-arginine produced unequivocal reversal of glucose-associated water and Na^+ absorption when perfused in the same intestinal segement but was without effect when perfused in jejunal or distal intestinal segments. These observations strongly suggest that circulating secretagogues are not a major mediator of the intestinal secretion induced by L-arginine, although a minor effect cannot be excluded with the methodology used in this study. The results also indicate that absorbed circulating L-arginine is not responsible for the secretion.

Phenothiazines inhibit intestinal secretion in rabbit[21] and human[21 22] intestine *in vitro*, inhibit cholera toxin induced jejunal secretion in the mouse *in vivo*,[2] and reduce stool fluid losses in human cholera.[23] They appear to act by an effect on intracellular calcium dependent regulator protein (calmodulin), which is postulated to be an important regulator of intestinal ion transport.[21 22] The inhibition by chlorpromazine of water and electrolyte secretion induced by L-arginine therefore suggests that L-arginine also acts *via* calmodulin. However, the cellular effects of chlorpromazine are protean and include membrane stabilisation,[24] displacement of membrane bound calcium,[25] and increased membrane permeability,[26] all of which could theoretically affect intestinal absorptive and secretory function. Preliminary experiments in our laboratory indicate that intestinal secretion induced by D-mannitol, which is generally considered to be purely osmotic in origin, is unaffected by chlorpromazine, suggesting that the effect of chlorpromazine on passive intestinal permeability is not likely to be of importance in its inhibition of L-arginine induced secretion. However, the precise cellular mechanism of L-arginine induced secretion in man is at present unknown. Further studies are being carried out to characterise the process.

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