Atrial natriuretic peptide and water and electrolyte transport in the human jejunum

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Abstract
The effects of atrial natriuretic peptide were investigated on water and electrolyte transport in the human jejunum. Six healthy male volunteers (aged 21–33 years) were studied using a triple lumen perfusion technique. A plasma like electrolyte solution containing polyethylene glycol (5 g/l) as a non-absorbable marker was perfused into the jejunum at 10 ml/min, and net water and electrolyte transport and transepithelial potential difference were measured. Subjects were studied single blind on two occasions with either intravenous atrial natriuretic peptide (6 pmol/min/kg for 90 minutes) or placebo (saline), both after controlled sodium intake over three days. Plasma atrial natriuretic peptide concentrations rose from (mean (SD)) 10·3 (3·6) pmol/l to a peak of 96·0 (61·8) pmol/l. Jejunal net water and electrolyte fluxes and potential difference were identical in both the atrial natriuretic peptide and the control studies. Compared with placebo atrial natriuretic peptide induced a significantly greater diuresis (peak 10·2 (6·0) v 1·8 (1·0) ml/min, $p < 0.05$) and natriuresis (peak 1069 (351) v 376 (208) μmol/min, $p < 0.01$) and haemoconcentration (haematocrit 0·405 (0·040) v 0·368 (0·018), $p < 0.01$). There was no difference in blood pressure, pulse rate, plasma electrolytes, and plasma osmolality between the two studies. There was no evidence to suggest an effect of atrial natriuretic peptide on jejunal water and electrolyte transport in healthy human subjects.

Atrial natriuretic peptide is a hormone which has been found in a variety of species.[1] The 126-amino acid propeptide is synthesised and stored in atrial myocytes. The most important biologically active natriuretic peptide released into the circulation is α atrial natriuretic peptide, the 99–126 fraction at the C-terminal end. In humans acute volume load with saline, high sodium intake, or conditions with increased extracellular volume such as chronic heart and renal failure, result in increased plasma atrial natriuretic peptide concentrations.[2] Intravenously infused synthetic human α atrial natriuretic peptide in normal subjects induces diuresis, natriuresis, and haemoconcentration,[3,4] can lower blood pressure,[5] and inhibits renin and aldosterone secretion.[6] Given these effects, it seems likely that atrial natriuretic peptide has a physiological role in the regulation of sodium balance and extracellular volume. Atrial natriuretic peptide receptors are found in various organs including the jejunum of the rat.[7,8] The effect of this hormone on small intestinal transport has not been extensively studied and the available data in

animals[8-11] and humans[12] are controversial. Our aim was therefore to investigate whether human atrial natriuretic peptide, infused intravenously in doses simulating pathophysiological blood concentrations, has an effect on water and electrolyte transport in the human jejunum using the triple lumen perfusion technique.

Methods
Six normal male volunteers aged 21–33 years (mean 24 years) were studied. Informed written consent was obtained from all subjects and the study protocol was approved by the Otago Hospital Board Ethics Committee.

STUDY PROTOCOL
Each subject was studied on two separate occasions at least one week apart, once with intravenous atrial natriuretic peptide and once with placebo. The subjects were on a specially composed diet with fixed sodium and potassium intakes of 150 and 70 mmol/day respectively for three days before each study. They were not admitted to hospital for this period. On the third day of the diet a 24 hour urine collection was made to measure electrolyte excretion and creatinine clearance. One subject did not complete the 24 hour collection on one occasion and all his urine baseline data for both tests were excluded from the final analysis. On the fourth day the volunteers were intubated with the intestinal perfusion tube at 0700 hours after an overnight fast. They then remained supine for the rest of the study, apart from brief periods of standing to pass urine. One intravenous cannula was inserted in each arm, one for blood sampling and the other for intravenous atrial natriuretic peptide/placebo infusion. A subcutaneous cannula was placed in one arm for potential difference measurements. The study consisted of a first control period (60 minutes, time −60 to 0), the active period (90 minutes, time 0 to 90), and a second control period (60 minutes, time 90 to 150). During the active period α human atrial natriuretic peptide or placebo (saline) was infused intravenously at 0·5 ml/min; α human atrial natriuretic peptide (Bachem, Torrance, CA) was dissolved, dispensed in 250 μg aliquots, and lyophilised as described previously.[3] Atrial natriuretic peptide vials (or the same volume of saline for control infusions) were reconstituted on the study day and made up to the appropriate volume in Haemaccel (Behringwerke AG, Marburg a.d. Lahn, Germany).[3] The effectively infused dose of hormone based on the assay of infusates was 6·2 (1·8) pmol/min/kg. Atrial natriuretic peptide and control infusions were randomised and performed single blind. Plasma electrolytes, plasma

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osmolality, and haematocrit were checked at time $-60, 0, 90$, and 150 minutes. Venous blood samples for measuring plasma atrial natriuretic peptide concentrations (drawn at 15 minute intervals during the infusion period and at 30 minute intervals before and after infusions) were collected on ice in tubes containing ethylene-diaminetetra-acetate. The samples were immediately spun at 4°C and stored at $-80°C$ until assayed. Urine samples were obtained at 30 minute intervals. After recording the volume, samples were taken to measure osmolality and electrolytes. One volunteer was unable to pass urine at the required times and was therefore excluded from that part of the analysis. Blood pressure and pulse rate were monitored at 15 minute intervals using an oscillometric, programmable blood pressure monitor with a digital display (Model 90202 Ambulatory Blood Pressure Monitor, SpaceLabs, Washington).

INTESTINAL PERFUSION
The subjects were intubated with a standard triple lumen perfusion tube[13] including a 15 cm mixing and 30 cm test segment. The infusion site was placed beyond the ligament of Treitz and its position was checked radiologically using an image intensifier. A plasma like electrolyte solution containing (mmol/l) Na^+ 140, K^+ 5, Cl^- 110, HCO_3^- 35 was perfused at 10 ml/min using a peristaltic pump (Harvard Apparatus, Millis, Mass). Polyethylene glycol (PEG 4000, 5 g/l) was added as non-absorbable volume marker. Fifteen minutes after starting the perfusion, aspirations from the proximal and distal aspiration points were started at 1 ml/min using syringe pumps (Sage, Model 351, Cambridge, Mass). The first 15 minute collection was discarded. After a further 30 minutes consecutive 15 minute collections were made for measurement of net water and electrolyte fluxes.[14] A 10 minute stagger period was allowed between proximal and distal aspirations. A sample of each aspirate was kept on ice and stored at $-80°C$ for measurement of cyclic guanosine monophosphate. The pH of the aspirates was measured immediately after collecting.

POTENTIAL DIFFERENCE
Intestinal transepithelial potential difference was measured as described previously.[15] It was measured continuously between the infusion tube, which served as flowing intraluminal electrode, and a subcutaneous reference electrode consisting of a saline filled plastic cannula inserted into the subcutaneous tissue of the right forearm. Intraluminal electrode and reference electrode were connected to an electrometer (Keithley Instruments, Cleveland, Ohio) via 3 M KCl agar
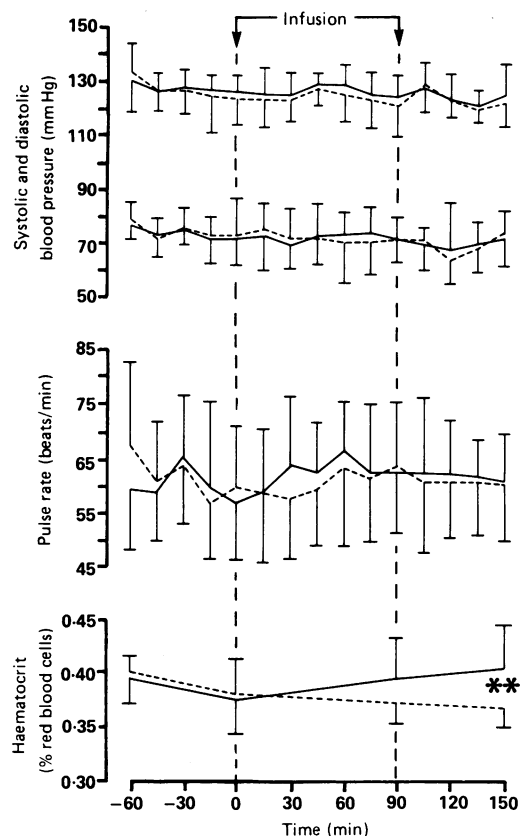

Figure 1: Mean (SD) arterial blood pressure, pulse rate, and haematocrit before, during, and after infusion of α human atrial natriuretic peptide (———) or placebo (saline, ———) in six normal men. **$p<0.01$.

bridges and Ag-AgCl electrodes. The potential difference was displayed on a chart recorder (Rikadenki, Tokyo, Japan). For technical reasons two subjects had recordings on only one occasion and were excluded from analysis.

ANALYTICAL METHODS
Sodium and potassium (Flamephotometer), chloride (Chloridometer), bicarbonate (acid back-titration), creatinine and haematocrit (Autoanalyser) were measured by standard laboratory techniques. Osmolality was measured by depression of freezing point (Fiske Osmometer, Uxbridge, Mass). Polyethylene glycol was measured using an improved turbidimetric method of Malawer and Powell.[16] Plasma immunoreactive atrial natriuretic peptide was measured in venous plasma extracts as previously described[1] except that a locally raised antiserum (R27)[17] was substituted for Peninsula antiserum and plasma samples were extracted using vycor glass powder (0·5 mg/1 ml plasma). Extraction efficiency was 85%. Cyclic guanosine monophosphate concentrations in the jejunal aspirates and urine were measured by radioimmunoassay using a commercially available kit (Amersham, TRK.500). Urine was diluted 1:10 with water before assaying and jejunal aspirates underwent an ethanol extraction procedure.

TABLE I Mean (SD) 24 hour urinary electrolyte excretion and creatinine clearance before atrial natriuretic peptide (ANP)/placebo studies (n=5)

	Na^+ (mmol/l)	K^+ (mmol/24 h)	Cl^- (mmol/l)	CrCl (ml/min)
ANP	158·0 (48·1)	80·1 (10·9) $p<0.05$	149·0 (26·8) $p<0.05$	117·3 (22·9)
Saline	135·0 (37·2)	62·6 (14·2)	121·4 (22·4)	107·6 (21·0)

STATISTICS
Results are expressed as mean (SD). Results from test and control studies were compared by

Figure 2: Mean (SD) plasma atrial natriuretic peptide concentrations before, during, and after infusion of a human atrial natriuretic peptide (O) or placebo (saline, ●) in six normal men.
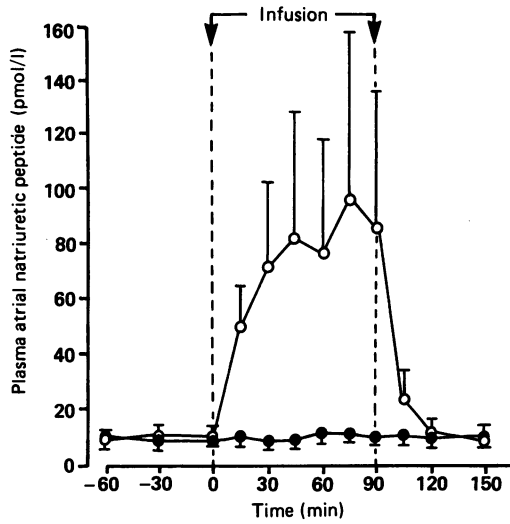
Student's t test for paired data. p values of <0·05 were regarded as significant.

Results

None of the subjects experienced any adverse effects during atrial natriuretic peptide infusion. The urinary electrolyte excretion was higher before the test days than before the control days (Table I). The difference was significant for potassium and chloride, but not for sodium. Renal function as assessed by creatinine clearance was identical before test and control days (Table I).

Figure 3: Mean (SD) urinary electrolyte and volume excretion before, during, and after infusion of a human atrial natriuretic peptide (■) or placebo (saline, □) in five normal men. $*p<0·05$, $**p<0·01$.

BLOOD PRESSURE, PULSE RATE, AND PLASMA VALUES (Figs 1 and 2)

There was no difference between atrial natriuretic peptide and control studies in systolic and diastolic blood pressure and pulse rate (Fig 1). Plasma osmolality and plasma electrolytes remained normal throughout the study and there was no difference between active and control days. While the haematocrit dropped slightly during the control studies, it steadily increased after starting the atrial natriuretic peptide infusion and was significantly higher at the end of the study (Fig 1). The plasma atrial natriuretic peptide concentrations remained within normal limits during the control studies and increased from 10·3 (3·6) to a peak of 96·0 (61·8) pmol/l 75 minutes after starting the atrial natriuretic peptide infusion (Fig 2); they returned to baseline values within 30 minutes of stopping the infusion.
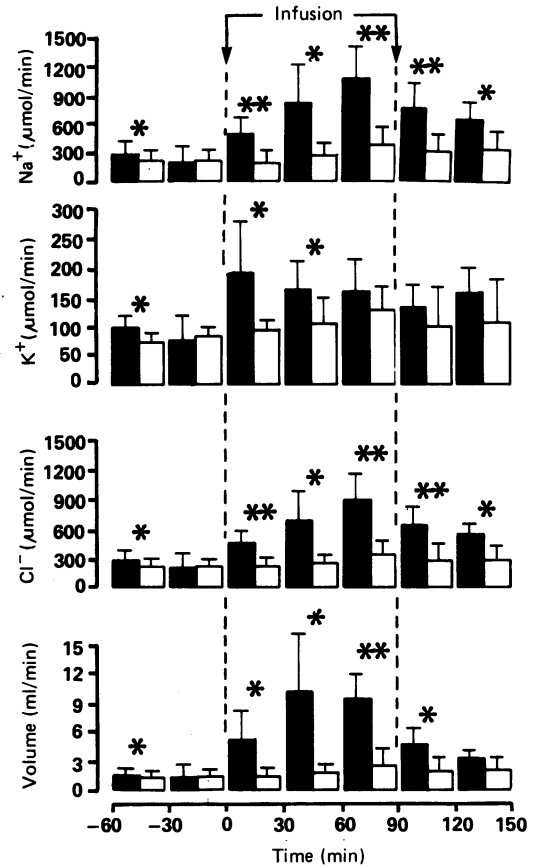
URINARY RESPONSE (Fig 3)

During the first 30 minutes of the experiments urinary electolyte excretion and diuresis were slightly but significantly higher on the α atrial natriuretic peptide days compared with the placebo days. There was no longer any difference during the second half hour of the first control period. Hormone infusion resulted in a fourfold increase of sodium and chloride excretion, which remained significantly higher for 60 minutes after stopping the infusion. Potassium excretion increased significantly in the first 60 minutes of the infusion. Urine volume increased sixfold and the diuretic effect was maintained for 30 minutes after completion of the infusion.

JEJUNAL WATER AND ELECTROLYTE TRANSPORT (Table II)

Net water and electrolyte fluxes, transepithelial

TABLE II   Net water and electrolyte transport, potential difference, and pH of aspirates in the human jejunum before, during, and after intravenous atrial natriuretic peptide (ANP)/placebo (saline) infusion (n=6, mean (SD))

	Control period (30 min)		Active period (90 min)		Control period (60 min)	
	ANP	Saline	ANP	Saline	ANP	Saline
ΔH₂O (μl/cm/min)	46·4 (17·6)	43·9 (25·4)	46·9 (13·0)	47·4 (37·3)	53·9 (42·3)	49·6 (20·1)
ΔNa⁺ (μmol/cm/min)	6·52 (2·45)	5·72 (3·74)	6·62 (1·65)	6·95 (5·36)	7·47 (6·25)	7·18 (3·02)
ΔK⁺ (μmol/cm/min)	0·31 (0·14)	0·29 (0·24)	0·31 (0·08)	0·31 (0·23)	0·33 (0·29)	0·31 (0·12)
ΔCl⁻ (μmol/cm/min)	3·44 (2·07)	2·98 (2·09)	3·26 (1·56)	3·35 (4·85)	3·98 (6·25)	3·67 (3·20)
ΔHCO₃⁻ (μmol/cm/min)	2·61 (1·03)	3·01 (0·96)	3·00 (0·74)	2·96 (0·82)	3·56 (0·70)	2·97 (0·51)
Potential difference (mV) (n=4)	−0·2 (1·4)	−0·9 (0·6)	0 (1·5)	−0·2 (1·1)	−0·7 (0·9)	−0·6 (0·9)
pH						
Proximal aspirates	7·03 (0·22)	7·02 (0·35)	7·05 (0·18)	6·99 (0·25)	7·08 (0·29)	7·10 (0·23)
Distal aspirates	6·78 (0·10)	6·79 (0·32)	6·71 (0·11)	6·68 (0·27)	6·71 (0·24)	6·70 (0·14)

TABLE III Mean (SD) jejunal c-GMP output (pmol/min) at the proximal aspiration point before, during, and after intravenous atrial natriuretic peptide (ANP) or placebo infusion (n=6)

| Aspirates | Minutes | | | |
	−15–0	30–45	75–90	135–150
ANP	3·7 (2·2)	3·5 (1·0)	4·8 (3·2)	2·8 (1·6)
Saline	3·4 (1·6)	3·5 (1·2)	4·2 (1·3)	4·3 (2·4)

potential difference, and pH of jejunal aspirates remained constant during the active period and both control periods, and no difference was observed between the results of the placebo and atrial natriuretic peptide studies.

CYCLIC GUANOSINE MONOPHOSPHATE (C-GMP) CONCENTRATIONS IN JEJUNAL ASPIRATES AND URINE (Table III)

c-GMP concentrations were measured in the proximal jejunal aspirates of the collections at −15–0, 30–45, 75–90, and 135–150 minutes. There was no difference in the output of c-GMP at the proximal aspiration site between atrial natriuretic peptide and control studies (Table III). In two subjects urinary c-GMP excretion was measured in the last 30 minute collection of the active period (60-90 minutes). During atrial natriuretic peptide infusion c-GMP excretion was higher than during placebo infusion (16·7 v 2·6 pmol/min and 28·1 pmol/min v 2·6 pmol/min).

Discussion

This study failed to show an effect of human α atrial natriuretic peptide on jejunal water and electrolyte transport when given in a dose aimed and shown to reproduce atrial natriuretic peptide plasma concentrations which are found in humans under pathophysiological conditions such as chronic renal failure[18] and chronic heart failure.[19] [20]

In the jejunum atrial natriuretic peptide receptors are localised in the mucosa beneath the columnar epithelium in the area of the villi but not the crypts,[7] [21] suggesting a possible influence on the absorptive properties of this intestinal segment. Published data on the effects of atrial natriuretic peptide on small intestinal transport are controversial. In rats some workers have shown increased[8] and some decreased[9] [22] effects on net absorption. In our own studies in rats[23] we showed that pharmacological doses (raising plasma concentrations by a factor of 130) had no effect on jejunal transport or colonic electrical parameters; however, even larger doses (raising plasma concentrations by a factor of over 1400) decreased net absorption and even induced secretion in the jejunum, but had no effect on the colon. This effect may well have been a non-specific or even 'toxic' response.

The only study of human transport, by Petritsch et al,[12] investigated both the jejunum and the ileum. The data gained from the jejunum are in agreement with our findings. In that study, however, the subjects were investigated on only one occasion and did not have a control perfusion with placebo infusion on a separate day. Since

perfusions constitute an intestinal volume load, this itself could stimulate endogenous atrial natriuretic peptide secretion. In our study plasma concentrations remained unchanged during control perfusions, indicating that the net intestinal saline load of 10 ml/min was not enough to release endogenous atrial natriuretic peptide. Petritsch et al[12] also reported similar plasma concentrations at the beginning and end of their perfusion period, which mitigates against appreciable volume load. It seems possible that more pronounced volume loading, especially together with the application of exogenous atrial natriuretic peptide, may decrease net intestinal absorption.

Blood volume expansion, a known stimulus to atrial natriuretic peptide release, decreased sodium and water absorption in the dog[24] and the rat.[9] [22] A c-GMP mediated atrial natriuretic peptide effect, previously reported in rats,[25] might be involved. In humans intravenous infusion increased plasma concentrations of c-GMP and increased urinary c-GMP excretion.[26] Tissue c-GMP released into the extracellular space[27] should be detectable in jejunal aspirates; however, we were unable to show differences in jejunal c-GMP output between atrial natriuretic peptide and control studies (Table III). In contrast, urinary c-GMP excretion increased sixfold to 11-fold during the diuresis accompanying atrial natriuretic peptide infusion (tested in two subjects). This provides convincing evidence of c-GMP mediated bioactivity of the infused atrial natriuretic peptide in the kidneys[26] but not in the gut.

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