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MODIFICATION OF RENAL AND TISSUE CATION TRANSPORT BY CHOLECYSTOKININ OCTAPEPTIDE IN THE RABBIT

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SUMMARY

1. Reports that gastric sodium loads cause a greater natriuresis than those administered intravenously, suggest that a gastric or portal sodium monitor exists which releases a humoral natriuretic factor. To determine whether cholecystokinin octapeptide (CCK-8) had direct renal natriuretic effects (and was therefore a candidate for this gut-derived natriuretic factor) we compared the natriuretic response to CCK-8 infused intravenously with that infused directly into the renal artery of six conscious male rabbits.

2. CCK-8 produced a significant log dose-dependent decrease in the fractional excretions of calcium ($P < 0{\cdot}05$) and magnesium ($P < 0{\cdot}005$) and a log dose-dependent increase in fractional sodium excretion ($P < 0{\cdot}025$). The significant decreases in the fractional excretions of calcium and magnesium were accompanied by log dose-dependent falls in their plasma levels (calcium, $P < 0{\cdot}05$, and magnesium, $P < 0{\cdot}005$), indicating movement of calcium and magnesium to extravascular sites. Studies of tissue calcium and magnesium levels in response to CCK-8 infusion showed that calcium accumulated in kidney and skeletal muscle.

3. We conclude that CCK-8 has direct renal natriuretic effects at the tubular level and could be the gut-derived natriuretic factor. In addition to its effects on sodium excretion, CCK-8 causes renal retention and increased gut absorption of calcium and magnesium with movement of these ions to extravascular sites.

INTRODUCTION

Many of the gut peptides have been shown to be ubiquitous in their distribution but only gastrin and secretin have physiologically defined functions. Cholecystokinin octapeptide (CCK-8) has long been suspected to be involved in fat digestion. It has been shown to be released in response to intraduodenal loads of fat and protein breakdown products (Ivy & Oldberg, 1928; Reeder, Becker, Smith, Rayford & Thompson, 1973; Walsh, Lamers & Valenzuela, 1982), and has been shown to cause pancreatic enzyme secretion and contraction of the gall-bladder with simultaneous relaxation of the sphincter of Oddi when infused. Recently these latter effects have

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been questioned as being pharmacological rather than physiological (Byrnes, Borody, Daskapoulos, Boyle & Bean, 1981; Walsh *et al.* 1982; Dockray, 1982). Thus it appears that the stimuli for CCK-8 release are well defined but the end-effects of this release are questionable.

Observations that sodium loads delivered orally or intraportally engender a greater natriuresis than those delivered intravenously (Daly, Roe & Horrocks, 1967; Passo, Thornborough & Rothballer, 1973; Lennane, Peart, Carey & Shaw, 1975*a*; Lennane, Carey, Goodwin & Peart, 1975*b*; Carey, Smith & Ortt, 1976; Carey, 1978) suggest that a humoral natriuretic factor originates in the gut. As protein foods (known stimulants of CCK release) have a moderate to high sodium content (Paul & Southgate, 1978), which must be dealt with simultaneously, CCK-8 may have a secondary role in sodium metabolism. We therefore sought to determine if CCK-8 could be considered a candidate for this gut natriuretic agent by a study in which the natriuretic response to CCK-8 infused intravenously and directly into the renal artery were compared.

The results of these infusion studies indicated that, in addition to affecting sodium excretion, CCK-8 influenced calcium and magnesium metabolism in such a way that these divalent cations were being moved out of the intravascular compartment, possibly into cells. To determine whether these ions were secreted into the gastrointestinal tract or taken up by tissues, CCK-8 was infused into anaesthetized rats and the calcium and magnesium levels in gastrointestinal perfusate and tissue samples were determined.

METHODS

(1) *Peptide infusion studies*

Six male New Zealand White rabbits which had undergone right nephrectomy and insertion of a permanent left renal artery catheter received stepped dose infusions of CCK-8 into either renal artery or the marginal ear vein (in randomized order) and the natriuretic responses were determined.

The left renal artery catheters were inserted under fentanyl (100 μg intramuscularly) and halothane (2·5% in oxygen, 3 l min^{-1}) general anaesthesia (Duggan, Macdonald & Rose, 1987) at least 1 week prior to experimentation. The catheters were maintained patent during this week by second daily flushes with 1 ml 0·9% saline.

On the day of experiment a self-retaining Foley's catheter (size 10F) was inserted per urethram, using lignocaine gel, and marginal ear vein and ear artery cannulae were inserted with lignocaine local anaesthesia. The rabbits were lightly restrained in restraint cages for the duration of the experiment. A lactate Ringer solution of the following composition (mM) was prepared: Na^+, 131; Cl^-, 111; K^+, 5; Ca^{2+}, 2; lactate. Each animal received an intravenous fluid load of this solution (10 ml kg^{-1} over 10 min) and a loading dose of [^{125}I]Hippuran (3 μCi, Amersham). They were then permitted a rest period of 1 h, during which [^{125}I]Hippuran was infused into the marginal ear vein cannula (0·03 μCi min^{-1}), and lactate Ringer solution into the renal artery, both infusions at a rate of 0·155 ml min^{-1}. A control infusion of vehicle alone of 30 min duration was followed by 30 min infusions of each dose of CCK-8 (10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 1·0, 10 and 100 pmol kg^{-1} min^{-1}) into either renal artery or marginal ear vein. When the definitive infusion was into the marginal ear vein, lactate Ringer solution was infused into the renal artery as a volume control. Blood pressure was monitored from the ear artery using a Bell and Howell pressure transducer and Neomedix Z200 chart recorder.

Urine was collected throughout, and blood was sampled at the end of each infusion period. Effective renal plasma flow (E.R.P.F.) was measured by [^{125}I]Hippuran clearance, and glomerular filtration rate (G.F.R.) by endogenous creatinine clearance. Plasma and urinary electrolytes were determined by atomic emission and absorption spectroscopy, osmolalities by freezing point depression and creatinine by autoanalyser acid picrate method.

(2) *Tissue calcium and magnesium levels*

Twelve male Sprague–Dawley rats weighing 350–550 g were randomly assigned to receive either vehicle alone (lactate Ringer solution) or vehicle plus CCK-8 (100 pmol kg^{-1} min^{-1}). They were then anaesthetized with 50 μg fentanyl and 20% urethane (0·5 ml per 100 g body weight) by intraperitoneal injection. A tracheostomy tube and a left carotid cannula were inserted through a ventral mid-line incision in the neck. The carotid catheter was introduced until its tip lay in the thoracic aorta so that a high dose of CCK-8 would reach the kidneys. The left femoral artery was cannulated to permit blood pressure monitoring and blood sampling. ^{47}Calcium chloride (2 μCi) was administered via the carotid artery cannula and lactate Ringer solution was infused at 0·03 ml min^{-1} for a 2 h rest equilibration period, at the end of which blood was sampled to determine plasma levels of ^{47}calcium and magnesium. Six rats then received CCK-8 plus vehicle (lactate Ringer solution) while the other six were given vehicle alone at 0·03 ml min^{-1} for 1 h, after which a second blood sample was taken. The rats were killed by barbiturate ovedose and samples of left kidney, left lobe of liver, skeletal muscle and bone were taken for determination of ^{47}calcium and magnesium levels.

Plasma magnesium levels were determined by atomic absorption spectrophotometry and tissue magnesium levels by modification of methods reported by Bhattacharya, Williams & Palmieri (1979), Battacharya, Goodwin & Crawford (1984) and Crawford & Bhattacharya (1985). Duplicate tissue samples were dried at 60 °C for 24 h and then weighed. Lipids were removed by extraction with diethyl ether and then digested with 1 ml 0·75 M-nitric acid at 80 °C for 24 h. The samples were centrifuged at 2500 r.p.m. for 5 min and the supernatant decanted; the pellet was resuspended in 1 ml 0·75 M-nitric acid and, after a second centrifugation, the two supernatants were combined and diluted to 5 ml with 0·75 M-nitric acid. This solution was then diluted 1 in 10 with 0·2% lanthanum chloride in 1% nitric acid and assayed by atomic absorption spectrophotometry in a lean air–acetylene flame with the cathode lamp set at 285·2 nm with a slit width of 0·5 nm.

(3) *Gastrointestinal secretion of calcium and magnesium*

Twelve male Sprague–Dawley rats weighing 350–550 g were randomly assigned to receive either vehicle alone (lactate Ringer solution) or vehicle plus CCK-8 (100 pmol $kg^{-1}min^{-1}$). They were then anaesthetized with 50 μg fentanyl and 20% urethane (0·5 ml per 100 g body weight) by intraperitoneal injection. A tracheostomy tube was inserted through a ventral mid-line incision in the neck. The abdomen was entered by a mid-line incision through the linea alba, the pelvic structures reflected and a polyethylene cannula (SP55) inserted via the left common iliac artery and advanced until its tip lay above the renal arteries. A polyvinyl catheter (SP74) was inserted in the duodenum just distal to the entry of the common bile duct and an efferent tube was inserted in the terminal ileum. The intestine was flushed with 30 ml 0·9% saline to achieve a clear effluent and thereafter perfused with saline in 15 ml h^{-1}. Vehicle alone was infused into the iliac catheter at 0·03 ml min^{-1} for 60 min, then either vehicle alone or vehicle plus CCK-8 for 60 min, followed by vehicle for a further 60 min. Intestinal effluent was collected for the last 5 min of each 15 min period and calcium and magnesium levels were determined.

Statistical methods

(1) *Peptide infusion studies.* Values obtained and derived values (renal vascular resistance, filtration fraction and fractional electrolyte excretions) at each infused dose were compared with those obtained during the control infusion using Student's t test P values of less than 0·05 being considered significant. The threshold dose was defined as that dose at which statistical significance ($P < 0·05$) was first attained.

These parameters were also assessed for linear trend with log dose of CCK-8 infused using a one-way t test of the slopes of individual rabbit responses (calculated by least squares). Again P values of less than 0·05 were considered significant.

(2) *Tissue calcium and magnesium levels.* The ^{47}calcium levels were calculated as counts ml plasma^{-1} and counts g tissue^{-1}. Efflux of ^{47}calcium from plasma was assessed as plasma counts at time zero (P_0) minus those after the 1 h infusion (P_1). For purposes of comparison, plasma and tissue counts were expressed as percentages of the plasma counts at the commencement of the infusion period (P_0). Control and peptide groups were compared by analysis of variance, with P values of less than 0·05 being considered significant.

Efflux of magnesium from plasma was calculated as magnesium concentration at commencement of the infusion period (P_0) minus that at its end (P_1). The CCK-8 and control groups were compared by analysis of variance. Tissue magnesium levels were expressed as mmol kg dry wt tissue^{-1} and compared by analysis of variance, with P values of less than 0·05 being considered significant.

(3) *Gastrointestinal secretion of calcium and magnesium.* The average rates of secretion of calcium and magnesium during each collection period for control and CCK-8 groups were compared by Student's t test, with P values of less than 0·05 being considered significant.

RESULTS

(1) *Peptide infusion studies*

Haemodynamic effects. Cholecystokinin octapeptide had no significant effects on systemic arterial pressure or pulse rate. There was no significant change from control values and no linear trend was observed with increasing dose of infused CCK-8. However, there were significant alterations in renal haemodynamics with both intravenous and renal artery infusions. Renal plasma flow fell significantly from control values commencing at a dose of 10 pmol kg^{-1} min^{-1} ($t = 1{\cdot}8485$, $P < 0{\cdot}05$) for renal infusion and at 10^{-1} pmol kg^{-1} min^{-1} ($t = 3{\cdot}2616$, $P < 0{\cdot}025$) for intravenous infusion (see Tables 1 and 2). The calculated renal vascular resistance increased significantly with threshold doses of 1·0 pmol kg^{-1} min^{-1} ($t = 2{\cdot}3525$, $P < 0{\cdot}025$) for renal administration and 10^{-1} pmol kg^{-1} min^{-1} ($t = 2{\cdot}6072$, $P < 0{\cdot}025$) for intravenous administration. Glomerular filtration rate did not differ significantly from control during CCK-8 infusion by either route.

Sodium excretion. Plasma sodium concentration did not change during CCK-8 infusion compared with control values. Total sodium excretion ($U_{Na}V$) increased significantly from control during CCK-8 infusion by the intrarenal route with a threshold dose of 10 pmol kg^{-1} min^{-1} ($t = 1{\cdot}9478$, $P < 0{\cdot}05$), whilst there was a non-significant increase during intravenous infusion (see Tables 1 and 2). Correction for glomerular filtration rate revealed a linear trend in the fractional sodium excretion which increased with increasing log dose of CCK-8 infused via the renal artery ($t = 2{\cdot}9239$, $P < 0{\cdot}025$; see Fig. 1). The change from control was apparent from the lowest infused dose of CCK-8, 10^{-4} pmol kg^{-1} min^{-1} ($P < 0{\cdot}05$). No significant change in fractional sodium excretion occurred during intravenous infusion.

Potassium excretion. There were no significant alterations in plasma potassium concentration, total potassium excretion or fractional potassium excretion during CCK-8 infusion by either the renal or intravenous route.

Calcium excretion. Plasma calcium decreased linearly with increasing log dose of CCK-8 infused by both renal and intravenous routes ($t = 4{\cdot}0497$, $P < 0{\cdot}005$ and $t = 3{\cdot}6592$, $P < 0{\cdot}01$ respectively). The threshold doses of CCK-8 were 10^{-1} pmol kg^{-1} min^{-1} for renal infusion ($t = 1{\cdot}9180$, $P < 0{\cdot}05$) and 10 pmol kg^{-1} min^{-1} for intravenous infusion ($t = 2{\cdot}1186$, $P < 0{\cdot}05$). Total calcium excretion showed a significant decrease from control during renal infusion at the highest dose infused, 100 pmol kg^{-1} min^{-1} ($t = 1{\cdot}8161$, $P < 0{\cdot}05$), while no significant change was observed during intravenous infusion. Correction for glomerular filtration rate showed a decrease in fractional calcium excretion from control values commencing at an infused dose of 10^{-3} pmol kg^{-1} min^{-1} ($t = 2{\cdot}062$, $P < 0{\cdot}05$), and a decreasing linear trend with increasing log dose of CCK-8 infused ($t = 2{\cdot}3865$, $P < 0{\cdot}05$) into the renal artery (see Fig. 1).

TABLE 1. Effects of intrarenal infusion of CCK-8 on mean arterial pressure (M.A.P.), renal plasma flow (E.R.P.F.), renal vascular resistance (R.V.R., arbitrary units), glomerular filtration rate (G.F.R.), plasma levels, total excretion (UV) and fractional excretions (F.e.) of sodium, calcium and magnesium. Values are means ± S.E.M. for $n = 6$ rabbits and were compared with control at each dose by Student's t test. * Indicates the dose at which statistical significance was first attained. A significant linear trend with increasing log dose of CCK-8 is indicated by †

		CCK-8 infused (pmol kg^{-1} min^{-1})						
	Control	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1·0	10	100
M.A.P. (mmHg)	73·56 ± 3·44	70·60 ± 4·29	70·40 ± 4·85	71·39 ± 4·10	73·39 ± 5·57	73·06 ± 5·12	73·11 ± 5·76	73·00 ± 6·95
R.V.R.	3·29 ± 0·92	7·08 ± 3·03	4·44 ± 0·87	7·51 ± 2·71	6·05 ± 2·22	9·36** ± 2·17	7·78 ± 2·64	5·90 ± 1·96
E.R.P.F. (ml min^{-1})	37·88 ± 12·67	27·77 ± 11·41	19·12 ± 8·12	22·77 ± 9·03	15·54 ± 3·65**	13·73 ± 3·10	11·89* ± 1·98	11·96 ± 4·66
G.F.R. (ml min^{-1})	6·23 ± 1·37	6·05 ± 2·31	5·97 ± 0·88	7·87 ± 1·52	7·51 ± 1·24	8·20 ± 1·72	8·81 ± 1·88	9·15 ± 1·30
$U_{Na}V$ (μmol min^{-1})	12·01 ± 2·87	21·51 ± 7·68	20·84 ± 4·00	21·54 ± 4·76	22·71 ± 6·71	25·54 ± 6·51	32·29* ± 8·93	33·40 ± 6·26
F.e. of Na (%)	1·03 ± 0·17	1·26** ± 0·28	1·71 ± 0·26	1·94 ± 0·27	1·76 ± 0·24	2·01 ± 0·25	2·29 ± 0·30	2·54†† ± 0·38
Plasma Ca (mmol l^{-1})	2·89 ± 0·08	2·88 ± 0·07	2·87 ± 0·08	2·79 ± 0·08	2·76* ± 0·06	2·68 ± 0·07	2·70 ± 0·04	2·69†† ± 0·08†
$U_{Ca}V$ (μmol min^{-1})	3·53 ± 0·43	2·85 ± 0·66	3·29 ± 0·87	2·83 ± 0·56	2·63 ± 0·62	2·65 ± 0·61	2·47 ± 0·47	2·45* ± 0·34
F.e. of Ca (%)	23·62 ± 3·99	19·06 ± 2·87	13·84* ± 1·19	13·81 ± 1·71	11·49 ± 1·06	11·84 ± 0·99	11·07 ± 0·84	9·91† ± 1·15
Plasma Mg (mmol l^{-1})	0·74 ± 0·02	0·72 ± 0·02	0·67 ± 0·01	0·69** ± 0·01	0·67 ± 0·01	0·62 ± 0·02	0·65 ± 0·02	0·63†† ± 0·01†
$U_{Mg}V$ (μmol min^{-1})	1·84 ± 0·31	2·09 ± 0·60	1·95 ± 0·48	1·58 ± 0·30	1·61 ± 0·51	1·40 ± 0·32	1·33 ± 0·26	1·33 ± 0·24
F.e. of Mg (%)	43·07 ± 4·54	44·53 ± 6·52	32·99 ± 4·67	29·53* ± 3·23	27·78 ± 4·71	27·62 ± 4·41	25·26 ± 3·98	24·56†† ± 5·45

* Denotes theshold dose: * $P < 0.05$; ** $P < 0.025$.

† Indicates linear trend with log dose: † $P < 0.05$, †† $P < 0.025$ and ††† $P < 0.005$.

TABLE 2. Effects of intravenous infusion of CCK-8 on mean arterial pressure (M.A.P.), effective renal plasma flow (E.R.P.F.), renal vascular resistance (R.V.R.), glomerular filtration rate (G.F.R.), plasma levels and total fractional excretions (F.e.) of sodium, calcium and magnesium. Values are means ± S.E.M. for $n = 6$ rabbits. Statistical analysis and determination of threshold dose–response are as for Table 1

		CCK-8 infused (pmol kg^{-1} min^{-1})						
	Control	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1·0	10	100
M.A.P. (mmHg)	73·05 ± 1·71	72·94 ± 1·20	73·45 ± 3·60	74·17 ± 1·49	70·89 ± 3·46	72·61 ± 2·68	71·20 ± 1·58	71·50 ± 1·71
R.V.R.	3·57 ± 0·66	12·75 ± 7·95	13·47 ± 7·15	10·94 ± 3·41	7·92** ± 1·37	8·05 ± 1·99	9·78 ± 3·31	6·97 ± 1·18
E.R.P.F. (ml min^{-1})	19·42 ± 2·79	15·30 ± 3·50	12·57 ± 2·66	7·87 ± 1·63	7·87** ± 0·73	8·37 ± 1·14	10·35 ± 2·12	9·88 ± 2·04
G.F.R. (ml min^{-1})	7·52 ± 1·16	6·05 ± 0·91	6·13 ± 1·24	6·44 ± 1·32	7·42 ± 1·12	6·97 ± 0·96	6·48 ± 1·03	8·01 ± 1·18
$U_{Na}V$ (μmol min^{-1})	25·74 ± 6·12	20·91 ± 4·86	19·50 ± 3·00	16·79 ± 3·47	18·86 ± 4·59	25·54 ± 6·36	14·51 ± 2·45	24·49 ± 3·90
F.e. of Na (%)	2·17 ± 0·44	2·29 ± 0·42	2·42 ± 0·39	1·96 ± 0·23	1·69 ± 0·23	2·77 ± 0·78	1·55 ± 0·14	2·46 ± 0·57
Plasma Ca (mmol l^{-1})	2·90 ± 0·11	2·84 ± 0·08	2·79 ± 0·10	2·82 ± 0·10	2·68 ± 0·11	2·66 ± 0·12	2·59* ± 0·08	2·66† ± 0·08
$U_{Ca}V$ (μmol min^{-1})	4·63 ± 1·91	2·86 ± 1·06	2·78 ± 0·89	2·16 ± 0·65	2·10 ± 0·67	3·53 ± 1·48	1·49 ± 0·54	2·99 ± 0·88
F.e. of Ca (%)	17·56 ± 5·57	16·57 ± 5·27	18·08 ± 5·32	13·12 ± 3·06	10·45 ± 2·89	19·47 ± 7·24	8·87 ± 2·99	13·62 ± 3·74
Plasma Mg (mmol l^{-1})	0·82 ± 0·03	0·76 ± 0·03	0·74 ± 0·04	0·74 ± 0·03	0·71 ± 0·05	0·70 ± 0·03	0·67 ± 0·03	0·63 ± 0·02
$U_{Mg}V$ (μmol min^{-1})	1·87 ± 0·58	1·51 ± 0·52	1·41 ± 0·42	1·16 ± 0·32	0·96 ± 0·17	1·13 ± 0·30	0·78 ± 0·19	1·15 ± 0·18
F.e. of Mg (%)	26·43 ± 4·89	31;36 ± 8·04	31·96 ± 7·59	25·45 ± 4·39	20·83 ± 3·90	20·26 ± 3·45	25·25 ± 8·40	25·57 ± 4·27

* Denotes threshold dose: $*P < 0.05$ and $**P < 0.025$.

† Indicates log dose-related linear trend, $P < 0.01$.

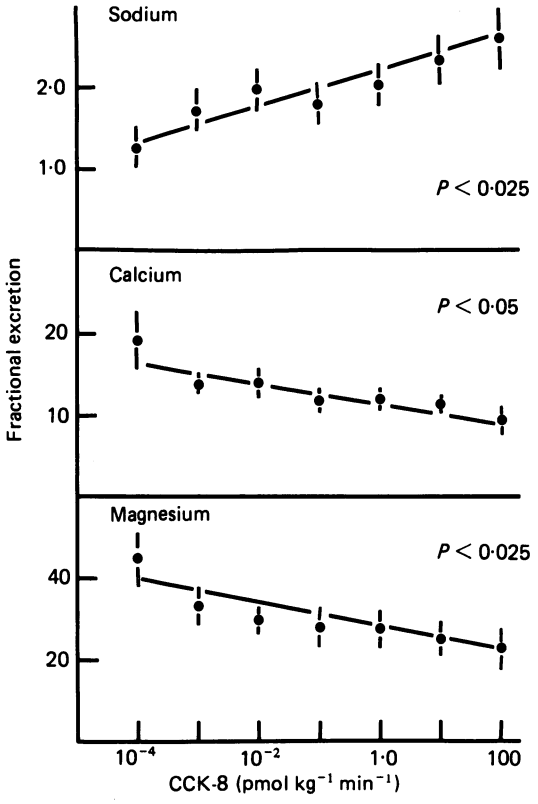

Fig. 1. Relationship of fractional excretions (expressed as a percentage) of sodium, calcium and magnesium to log dose of cholecystokinin octapeptide infused into the renal artery. Values depicted are mean ± standard error of mean for $n = 6$ rabbits. P value refers to log dose-related linear trend.

TABLE 3. Tissue ^{47}calcium levels. P_0 and P_1 are counts ml plasma^{-1} at the beginning and the end of the infusion period. Tissue radioactivity levels measured as counts mg tissue^{-1} have been expressed as percentages of P_0. Responses to CCK-8 and to control infusions were compared by analysis of variance ($n = 6$)

	Control	CCK-8
$P_0 - P_1/P_0$	21·88 ± 2·37	27·03 ± 2·30
Kidney (% of P_0)	23·95 ± 5·55	32·11** ± 5·64
Liver (% of P_0)	40·23 ± 10·92	29·27 ± 5·30
Muscle (% of P_0)	1·70 ± 0·34	4·73* ± 0·78
Bone (% of P_0)	6·91 ± 1·10	10·54 ± 1·35

*$P < 0·05$, **$P < 0·005$.

Magnesium excretion. There was a significant decrease in plasma magnesium concentration from control during CCK-8 infusion with threshold doses of 10^{-2} pmol kg^{-1} min^{-1} ($t = 2·3333$, $P < 0·025$) for intrarenal infusion and 1·0 pmol kg^{-1} min^{-1} ($t = 2·2827$, $P < 0·025$) for intravenous infusion. This decrease showed dose dependence, with a decreasing linear trend on renal infusion ($t = 5·0020$, $P < 0·005$; Table 1). Total magnesium excretion ($U_{Mg}V$) displayed only non-significant decreases

from control during both renal and intravenous infusions. A log dose-related fall in fractional magnesium excretion during renal infusion was found on analysis for linear trend ($t = 2{\cdot}7639$, $P < 0{\cdot}025$; Fig. 1), with a threshold dose of 10^{-2} pmol kg^{-1} min^{-1} ($t = 2{\cdot}2167$, $P < 0{\cdot}05$).

Free water clearance. No significant trends in free water clearance were observed with either intrarenal or intravenous infusion of CCK-8.

(2) *Tissue calcium and magnesium levels*

Although a greater efflux of ^{47}calcium occurred from the intravascular compartment during CCK-8 infusion than during control infusion (parallelling the changes observed in the rabbit) the difference did not achieve statistical significance.

TABLE 4. Tissue magnesium levels. P_0 and P_1 are plasma magnesium levels at the beginning and end of the infusion period. Tissue and plasma magnesium levels for CCK-8 and control groups were compared by analysis of variance ($n = 6$)

	Control	CCK-8
$P_0 - P_1$(mmol l^{-1})	0·05 ± 0·02	0·16* ± 0·06
Kidney (mmol kg dry wt^{-1})	35·92 ± 2·02	34·34 ± 2·94
Liver (mmol kg dry wt^{-1})	35·59 ± 1·16	34·17 ± 0·56
Muscle (mmol kg dry wt^{-1})	45·67 ± 2·03	42·17 ± 1·33
Bone (mmol kg dry wt^{-1})	184·70 ± 5·88	174·60 ± 2·92

*$P < 0{\cdot}025$.

However, there were significant differences between CCK-8 and control infusions in the uptake of ^{47}calcium to tissues. Levels of ^{47}calcium in the kidney increased from 23·95 ± 2·37 for control infusion to 32·11 ± 5·64 for CCK-8 infusion ($P < 0{\cdot}005$), and skeletal muscle from 1·70 ± 0·34 for control to 4·73 ± 0·78 for CCK-8 ($P < 0{\cdot}05$), whilst the decrease in hepatic uptake just failed to attain significance ($P < 0{\cdot}08$; see Table 3).

The decrease in plasma magnesium levels was greater during CCK-8 infusion than during control infusion ($P < 0{\cdot}025$; Table 4), again parallelling the changes observed in the rabbit. Although all of the tissues sampled showed decreases in magnesium content in response to CCK-8 infusion compared with control, in no instance did these decreases attain statistical significance.

(3) *Gastrointestinal secretion of calcium and magnesium*

During the period of CCK-8 infusion, significant decreases in the secretion of calcium and magnesium into the gastrointestinal effluent were observed compared with the control group (Fig. 2). In the third 60 min period, the calcium secretion returned to control values while magnesium secretion increased significantly before returning to control.

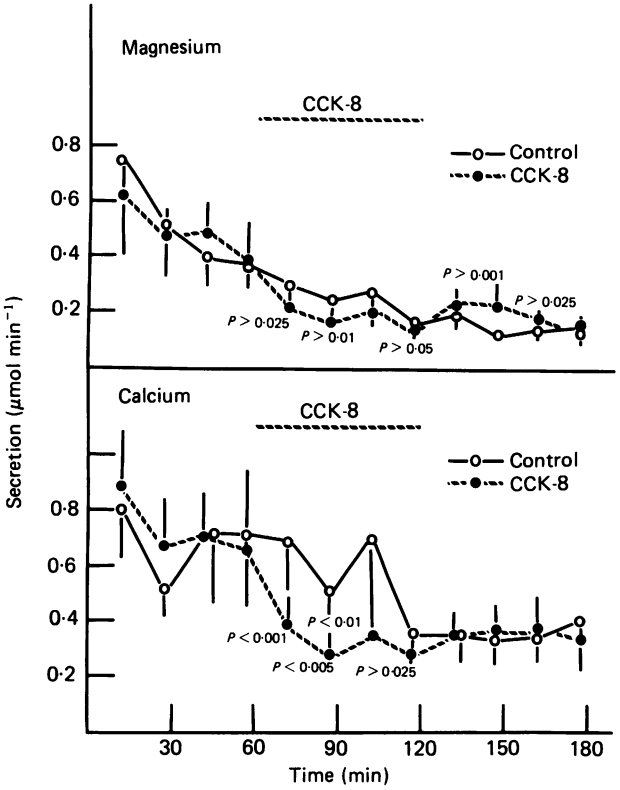

Fig. 2. Gastrointestinal magnesium (upper panel) and calcium (lower panel) secretion rates. Values are means ± S.E.M. and represent an average rate of secretion for the final 5 min of each 15 min period. Vehicle alone was infused in each group from 0 to 60 min. CCK-8 (100 pmol kg^{-1} min^{-1}) or vehicle alone was infused from 60 to 120 min and vehicle only in both groups from 120 to 180 min. Values during CCK-8 infusion were compared with control values for each collection period using Student's t test.

DISCUSSION

From this study it appears that cholecystokinin octapeptide has significant direct renal effects. Renal artery infusions resulted in significant alterations in both renal haemodynamics and cation excretion, while intravenous infusions over the same dose range only tended to affect the renal haemodynamics. The haemodynamic changes observed, i.e. a fall in renal plasma flow with no significant change in glomerular filtration rate, indicate that CCK-8 acts as a vasoconstrictor on the efferent arteriole. However, this effect is most probably a pharmacological rather than a physiological one, as the concentration of CCK-8 reaching the kidney on renal infusion by wrought estimation (as calculated by infused dose × effective renal plasma flow), is 10–20 times those reported by Walsh, Lamers & Valenzuela (1982), Calam, Ellis & Dockray (1982) and Dockray (1982) following meal stimulation.

The increase in urinary sodium excretion appears to be a result of actions on the renal tubules by CCK-8 since fractional sodium excretion showed an increase with

increasing log dose of CCK-8 when infused into the renal artery. This may be mediated at more than one site in the nephron, as the fractional excretions of both calcium and magnesium fell while the sodium fractional excretion rose in response to CCK-8 infusion. Cholecystokinin octapeptide probably acts, therefore, on the sodium–calcium exchange pump in the basolateral membrane of proximal tubular cells, causing the simultaneous retention of calcium and excretion of sodium. However other sites of action of CCK-8 along the nephron promoting calcium reabsorption cannot be excluded: Lassiter, Gottschalk & Mylle (1963), Bourdeau & Burg (1979), Jamison, Frey & Lacy (1974) and De Rouffignac, Morel, Moss & Roniel (1973) have shown that calcium reabsorption occurs in the pars recta, thick ascending limb of the loop of Henle and the distal convoluted tubule. As calcium absorption at these sites appears to be independent of sodium excretion, they cannot be considered as the only site of action of CCK-8.

Magnesium has also been shown by micropuncture studies to be reabsorbed in the proximal and distal tubules as well as the thick ascending limb of the loop of Henle (Massry, Coburn & Kleeman, 1969; Morel, Roniel & Le Grimellec, 1969; Wen Evanson & Dirks, 1970; Brunette, Vigneault & Carriere, 1974; Quamme, Wong, Dirks, Roniel De Rouffignac & Morel 1978) with most of the filtered magnesium being reabsorbed in the latter. The transport mechanisms at these sites have not been elucidated, though active transport mechanisms (as opposed to voltage-dependent ones) have been implicated (Le Grimellec, Roniel & Morel, 1973, 1974; Massry, Ahumada, Coburn & Kleeman, 1979; Quamme & Dirks, 1980). It is quite conceivable therefore that at one or more of these sites magnesium may be absorbed in exchange for other cations (such as sodium), thus explaining the observed increase in fractional sodium excretion and simultaneous decrease in fractional magnesium excretion with increasing dose of CCK-8 infused.

These effects on the fractional excretions of sodium, calcium and magnesium are apparent from doses which result in concentrations of CCK-8 reaching the kidney of 0·01–1·21 pmol l^{-1} (by wrought estimation). These lie within reported resting plasma ranges (Byrnes *et al.* 1981; Walsh *et al.* 1982; Calam *et al.* 1982) for CCK-8, and therefore control of cation homeostasis may represent another potential physiological function for CCK-8.

The combination of decreasing plasma levels of calcium and magnesium, taken together with the decreases in their excretions, suggest that CCK-8 caused an efflux of these ions from the intravascular space, and possibly increased their intracellular concentrations. The changes in ^{47}calcium levels in tissues in response to CCK-8 infusion compared with control suggest that this accumulation occurred in kidney and skeletal muscle, while the studies on gastrointestinal effluent suggest that CCK-8 stimulates calcium absorption. In the case of magnesium our study showed only that it did not accumulate in any of the tissues sampled, and was not secreted into the gut in response to CCK-8 infusion.

We conclude therefore that CCK-8 affects sodium excretion by tubular mechanisms at doses which lie within reported physiological ranges and therefore may be considered a possible candidate for the gut-derived humoral natriuretic factor implied by the studies of sodium excretion in response to gut and intravenous sodium

loads. In addition, CCK-8 reduces urinary excretion of calcium and magnesium and appears to facilitate the accumulation or transport of calcium into kidney and skeletal muscle.

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REFERENCES

BHATTACHARYA, S. K., WILLIAMS, J. C. & PALMIERI, G. M. A. (1979). Determination of calcium and magnesium in cardiac and skeletal muscle by atomic absorption spectroscopy using a stoichiometric nitrous oxide–acetylene flame *Analytical Letters* **12**, 1451–1475.

BHATTACHARYA, S. K., GOODWIN, T. G. & CRAWFORD, A. J. (1984). Submicro determination of copper and zinc in needle biopsy-sized cardiac and skeletal muscles by atomic absorption spectroscopy using stoichiometric air–acetylene flame. *Analytical Letters* **17**, 1567–1591.

BOURDEAU, J. E. & BURG, M. B. (1979). Voltage dependence of calcium transport in the thick ascending limb of Henle's loop. *American Journal of Physiology* **236**, F357–364.

BRUNETTE, M. G., VIGNEAULT, N. & CARRIERE, S. (1974). Micropuncture study of magnesium transport along the nephron in the young rat. *American Journal of Physiology* **227**, 891–896.

BYRNES, D. J., BORODY, T., DASKAPOULOS, G., BOYLE, M. & BENN, I. (1981). Cholecystokinin and gall bladder contraction: effect of CCK infusion. *Peptides–Fayetteville New York–Ankho International Incorporated* **2**, suppl. 2, 259–262.

CALAM, J., ELLIS, A. & DOCKRAY, G. J. (1982). Identification and measurement of molecular variants of cholecystokinin in duodenal mucosa and plasma. *Journal of Clinical Investigation* **69**, 218–225.

CAREY, R. M., SMITH, J. R. & ORTT, E. M. (1976). Gastrointestinal control of sodium excretion in sodium-depleted conscious rabbits. *American Journal of Physiology* **230**, 1504–1508.

CAREY, R. M. (1978). Evidence for a splanchnic sodium input monitor regulating renal sodium excretion in man. *Circulation Research* **43**, 19–24.

CRAWFORD, A. J. & BHATTACHARYA, S. K. (1985). Microanalysis of copper and zinc in biopsy-sized tissue specimens by atomic absorption spectroscopy using a stoichiometric air–acetylene flame. In *Varian Instruments at Work* **AA-46**. Mulgrave, Victoria: Varian Instruments.

DALY, J. J., ROE, J. W. & HORROCKS, P. (1967). A comparison of sodium excretion following the infusion of saline into systemic and portal veins in the dog: evidence for a hepatic role in the control of sodium excretion. *Clinical Science* **33**, 481–487.

DE ROUFFIGNAC, C., MOREL F., MOSS, N. & RONIEL, N. (1973). Micropuncture study of water and electrolyte movements along the loop of Henle in *Psammomys*, with special reference to magnesium, calcium and phosphorus. *Pflügers Archiv* **344**, 309–326.

DOCKRAY, G. J. (1982). The physiology of cholecystokinin in brain and gut. *British Medical Bulletin* **38**, 253–258.

DUGGAN, K. A., MACDONALD, G. J. & ROSE, M. A. (1987), Chronic renal artery catheterization in the conscious rabbit. *Laboratory Animal Science* (in the Press).

IVY, A. C. & OLDBERG, E. (1928). A hormone mechanism for gall-bladder contraction and evacuation. *American Journal of Physiology* **86**, 599–613.

JAMISON, R. L., FREY, N. R. & LACY, F. B. (1974). Calcium reabsorption in the thin loop of Henle. *American Journal of Physiology* **227**, 745–751.

LASSITER, W. E., GOTTSCHALK, C. W. & MYLLE, M. (1963). Micropuncture study of renal tubular reabsorption of calcium in normal rodents. *American Journal of Physiology* **204**, 771–775.

LE GRIMELLEC, C., RONIEL, N. & MOREL, F. (1973). Simultaneous Mg, Ca, P, K, Na and Cl analysis in rat tubular fluid. I. During perfusion of either inulin or ferrocyanide. *Pflügers Archiv* **340**, 181–196.

LE GRIMELLEC, C., RONIEL, N. & MOREL, F. (1974). Simultaneous Mg, Ca, P, K, Na and Cl analysis in rat tubular fluid. III. During acute Ca plasma loading. *Pflügers Archiv* **346**, 171–188.

LENNANE, R. J., PEART, W. S., CAREY, R. M. & SHAW, J. (1975*a*). A comparison of natriuresis after oral and intravenous sodium loading in sodium-depleted rabbits: evidence for a gastrointestinal or portal monitor of sodium intake. *Clinical Science and Molecular Medicine* **49**, 433–436.

LENNANE, R. J., CAREY, R. M., GOODWIN, T. J. & PEART, W. S. (1975*b*). A comparison of natriuresis after oral and intravenous sodium loading in sodium-depleted man: evidence for a gastrointestinal or portal monitor of sodium intake. *Clinical Science and Molecular Medicine* **49**, 437–440.

MASSRY, S. G., COBURN, J. W. & KLEEMAN, C. R. (1969). Magnesium handling in the dog. *American Journal of Physiology* **216**, 1460–1467.

MASSRY, S. G., AHUMADA, J. J., COBURN, J. W. & KLEEMAN, C. R. (1970). Effect of $MgCl_2$ infusion on urinary Ca and Na during reduction in their filtered loads. *American Journal of Physiology* **219**, 881–885.

MOREL, F., RONIEL, N. & LE GRIMELLEC, C. (1969). Electron probe analysis of tubular fluid composition. *Nephron* **6**, 350–364.

PASSO, S. S., THORNBOROUGH, J. R. & ROTHBALLER, A. B. (1973). Hepatic receptors in control of sodium excretion in anaesthetised cats. *American Journal of Physiology* **224**, 373–375.

PAUL, A. A. & SOUTHGATE, D. A. T. (1978). In *McChance and Widdowson's 'The Composition of Foods'*, 4th revised and extended edition of MRC special report No. 297, pp. 37–273. London: Her Majesty's Stationery Office.

QUAMME, G. A., WONG, N. L. M., DIRKS, J. H., RONIEL, N., DE ROUFFIGNAC, C. & MOREL, F. (1978). Magnesium handling in the dog kidney. *Pflügers Archiv* **377**, 95–99.

QUAMME, G. A. & DIRKS, J. H. (1980). Intraluminal and contraluminal magnesium on magnesium and calcium transport in the rat nephron. *American Journal of Physiology* **238**, F187–198.

REEDER, D. R., BECKER, H. D., SMITH, N. J., RAYFORD, P. L. & THOMPSON, J. C. (1973). Measurement of endogenous release of cholecystokinin by radioimmunoassay. *Annals of Surgery* **178**, 304–310.

WALSH, J. H., LAMERS, C. B. & VALENZUELA, J. E. (1982). Cholecystokinin-octapeptide immunoreactivity in human plasma. *Gastroenterology* **82**, 438–444.

WEN, S. F., EVANSON, R. L. & DIRKS, J. H. (1970). Micropuncture study of magnesium transport in proximal and distal tubule of the dog. *American Journal of Physiology* **219**, 570–576.