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Neurotensin and antinatriuresis in the conscious rabbit

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The mechanism of alteration in renal sodium excretion in response to dietary changes is complex and poorly understood. A gut 'sensor' might exist which regulates the renal response and this may involve one or more of the now ubiquitous gastrointestinal peptides. Several of these gut peptides, including neurotensin, have been found within the kidney. Plasma levels of neurotensin, which is both a circulating hormone and putative neurotransmitter, rise promptly on feeding. When infused into the conscious rabbit, neurotensin produces a dose-related fall in renal sodium excretion.

Introduction Many studies concerned with establishing or repudiating a role for the gastrointestinal tract in the regulation of sodium excretion have concerned themselves with the degree of natriuresis following an oral versus an intravenous saline load (Lennane, Peart, Carey & Shaw, 1975; Lennane, Carey, Goodwin & Peart, 1975; Carey, Smith & Ortt, 1976; Gordon & Peart, 1979; Hanson, McLane-Vega, Childers, Gleason & Schneider, 1980; Obika, Fitzgerald, Gleason, Zucker & Schneider, 1981). Few have addressed themselves to the mechanism of antinatriuresis following sodium deprivation and even among these there is no clear consensus (Anderson & Linas, 1978). The initial rapid fall in renal sodium excretion cannot be explained by changes in plasma electolytes, osmolality, protein concentration, extracellular fluid volume or glomerular filtration rate (Anderson & Linas, 1978; Gordon, James, Peart & Wilson, 1978; Moss, Gordon, Forsling, Peart, James & Roddis, 1981). One or a combination of gut peptides may be part of the signal influencing renal sodium excretion in response to dietary changes. Many are putative neurotransmitters within the central and peripheral nervous systems as well as circulating gastrointestinal hormones (Larsson, 1980; Dockray & Gregory, 1980).

We describe here, the effect of one such peptide, neurotensin, on renal sodium excretion in the conscious rabbit. Intravenous infusion of doses which achieved plasma levels found after feeding in man (Mashford, Nilsson, Rokaeus & Rosell, 1978), produced a significant reduction in renal sodium excretion and we conclude that neurotensin could be involved in sodium homeostasis.

Methods Four male sandy half-lop rabbits from the same litter were studied. Each received 3 different doses of synthetic neurotension (Cambridge Research Biochemicals), 2, 20 and 200 pmol kg^{-1} min^{-1} by intravenous infusion and one control (vehicle only) infusion on 4 separate occasions, at weekly intervals. Infusion order was determined by a randomized Latin square design. The animals were fed a standard laboratory diet (RHM R14, Labsure Animal Diets) and water *ad libitum* until 3 h before the start of each infusion. On the day of the study, cannulations of an ear artery and vein were performed, under lignocaine local anaesthesia and a self-retaining catheter inserted *per urethram* to allow free drainage and collection of urine. After 1 h of rest, the animals received a 'water load' as 25 ml kg^{-1} of intravenous 0.28 M glucose solution (isotonic) over 20 min. This was followed by infusion of the radiolabelled (Amersham International, UK) substances [^{125}I]-hippuran (1.48 kBq min^{-1}) and [^{51}Cr]-ETDA (0.74 kBq min^{-1}) in 0.28 M glucose to enable estimation of effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) by clearance respectively and a continuous infusion of 0.28 M glucose (0.6 ml min^{-1}) to promote urine flow. A further 20 min elapsed before starting the first of eight, 20-min urine collections. There was a 10 min interval between each collection. The bladder was flushed with 5 ml deionised water at the end of each collection and with 10 ml at the end of each interval, the latter being discarded. Continuous recordings of arterial blood pressure and heart rate were made and blood samples were taken at the midpoint of each collection. Infusion of neurotensin or the control infusate was begun 10 min before the start of the third urine collection and stopped at the end of the sixth collection. A single blood sample was taken between the fifth and sixth collections for estimation of plasma neurotensin.

Electrolytes were determined by flame photometry, osmolality by freezing point depression, plasma solids by drying weight samples to a constant weight at 120°C, plasma renin activity (PRA) and plasma

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Table 1 Effects of neurotensin on plasma and other variables in conscious rabbits

	Control	*Neurotensin infusion* 2 pmol kg^{-1} min^{-1}	20 pmol kg^{-1} min^{-1}	200 pmol kg^{-1} min^{-1}	
^{P}NTLI (pmol l^{-1})	<3	15±2	117±22	1165±91	(4)
MBP (mmHG)	60±2	62±4	58±4	64±3	(12)
HR (beats min^{-1})	271±9	243±7	262±8	242±5	(12)
P_{Na} (mmol l^{-1})	137±1	136±1	134±1	134±1	(12)
P_K (mmol l^{-1})	3.6±0.1	3.5±0.1	3.5±0.1	3.4±0.1	(12)
P_{Osm}(mOsm kg^{-1})	275±1	278±2	276±2	275±2	(12)
PS (%)	7.68±0.20	7.22±0.16	7.35±0.14	7.59±0.26	(8)
†PRA (pg ml^{-1} h^{-1})	12.9±1.9	13.6±2.4	17.9±3.2	*22.8±3.7	(8)
ERPF (ml min^{-1})	54.6±4.9	54.7±4.8	48.7±2.8	42.6±3	(12)
GFR (ml min^{-1})	11.0±0.9	10.9±1.0	9.6±0.9	*7.9±0.8	(12)
†$\dot{V}$ (µl min^{-1})	362.6±96.6	171.7±49	167.5±47.6	*78.9±12.3	(12)
†$U_{Na}V$ (µmol min^{-1})	9.6±1.4	*4.0±1.3	*4.9±1.2	*1.9±1.2	(12)
†U_KV (µmol min^{-1})	9.7±1.1	8.7±1.0	7.2±0.9	*5.3±0.6	(12)
†FENa (%)	0.67±0.17	*0.29±0.06	0.40±0.10	*0.19±0.04	(12)
†FEK (%)	25.7±3.2	24.0±2.1	23.0±3.3	21.1±2.2	(12)

Values based on observations made during the fourth, fifth and sixth urine collections. Mean ± s.e., number (n) in parentheses. *Significantly different from control ($P<0.05$). †Transformed variable. P_{NTLI} (plasma neurotensin-like immunoreactivity), P_{Na} (plasma sodium), P_K (plasma potassium); other abbreviations in text.

neurotensin by radioimmunoassay (Sever, Peart, Davies, Tunbridge & Gordon, 1979; Blackburn & Bloom, 1979). Data were analysed by a three-way analysis of variance for repeated measurements in the same animal, with weight and time as covariates, followed by multiple comparisons with the control (Dunnett, 1964). Geometric means and approximate standard errors are quoted for those variables requiring logarithmic transformation to approximate normality.

Results (Table 1) There was no effect of neurotensin on mean blood pressure (MBP), heart rate (HR), ERPF, filtration fraction (FF, ratio of GFR : ERPF), plasma electrolytes, plasma osmolality (P_{Osm}) or plasma solids (PS, a measure of plasma protein concentration) at the doses infused. Although changes in PRA, GFR and urine flow rate ($\dot{V}$) occurred at the highest dose infused, the fall in renal sodium excretion ($U_{Na}V$) was significant even at the low and middle doses, with plasma neurotensin levels within the human postprandial range. The fractional excretion of sodium (FENa), expressed as a percentage of the filtered load (product of plasma sodium concentration and GFR), also fell during neurotensin infusion, but the fall was only significant during infusion of the low and high doses. There was no significant change in the fractional excretion of potassium (FEK) at any dose.

Discussion Originally, neurotensin was found to produce vasodilatation and hypotension in the rat (Carraway & Leeman, 1973), but has since been shown to have no effect on blood pressure or heart rate in doses of 10–20 pmol kg^{-1} min^{-1} (Rosell, Burcher, Chang & Folkers, 1976). Thus it seems unlikely that the changes we observed were due to haemodynamic effects of neurotensin at the low and middle doses, although distributional changes within the kidney cannot be excluded. Neurotensin is thought to interact with dopaminergic systems and to behave as a dopamine antagonist (Brown & Miller, 1982). Dopamine binding sites are present in renal tissue (Roberts, Woodruff & Poat, 1977) and dopamine is known to be natriuretic (Lee, 1982). Therefore, it is conceivable that neurotensin alters dopaminergic activity peripherally and perhaps even centrally. It may influence sympathetic nervous function and release of other hormones such as prolactin (Brown & Vale, 1977), thus affecting $U_{Na}V$, GFR and PRA (Bliss & Lote, 1982). Furthermore, neurotensin immunoreactivity has been demonstrated within renal peptidergic nerves (Forssmann, Hock & Metz, 1982) and neurotensin may act directly to alter intrarenal blood flow or tubular function.

The derived variable of fractional excretion minimizes the changes in $U_{Na}V$ due to alterations in GFR. Thus, the fall in $U_{Na}V$ during infusion of the middle dose seems largely due to the small reduction in GFR. However, the decrease in FENa during

infusion of the low and high doses seems to suggest a direct tubular action of neurotensin, for even at the high dose, the reduction in GFR does not fully explain the fall in $U_{Na}V$. The fact that the relationship is not strictly dose-dependent may simply indicate a changing balance between haemodynamic and tubular effects of neurotensin.

The rise in PRA seen at the high dose infused could be the result of macula densa stimulation due to reduced sodium delivery, although the exact nature of the stimulus perceived, if any, by the macula densa is uncertain (Davis & Freeman, 1976). The finding that renal potassium excretion (U_KV) did not change until the fall in filtered load (GFR) may reflect increased distal tubular Na^+/K^+ exchange. The small fall in GFR at pharmacological concentrations of plasma neurotensin, may be a direct effect or secondary to some other factor such as increased local production of angiotensin-II.

Finally, neurotensin may influence yet another aspect of sodium homeostasis, that of sodium appetite. Like some other neuropeptides, it seems to be involved in the regulation of appetite (Koopmans, 1981), and this might include sodium appetite. Therefore, it seems possible that neurotensin could play a role in the control of sodium balance and that further studies, particularly in man are indicated.

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