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RENAL MECHANISMS OF HUMAN α-ATRIAL NATRIURETIC PEPTIDE IN MAN

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SUMMARY

1. Eight normal volunteers were studied on two separate days after being dehydrated overnight. Each volunteer received a background intravenous infusion of arginine vasopressin ($5{\cdot}5 \times 10^{-7}$ i.u. kg^{-1} min^{-1}) on both days and also received an intravenous infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) plus carrier on one day and carrier alone on the other. The ensuing changes in blood pressure, in the excretion of urinary solutes, and in the excretion of solute-free water were recorded.

2. The infusion of atrial peptide had a small hypotensive effect, and increased the rate of excretion of sodium but not of potassium. There were no significant changes of urinary osmolality or of creatinine clearance.

3. The infusion of atrial peptide increased the rate of solute-free water reabsorption and did so in direct proportion to its effect of increasing sodium excretion.

4. A further six normal, dehydrated volunteers were studied on each of two days after taking 500 mg of lithium carbonate on the previous evening. On one day, they received an intravenous infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) plus carrier and on the other day they received carrier alone. The excretion of urinary electrolytes and the creatinine clearance were recorded.

5. The infusion of atrial peptide produced significant increases in the rates of excretion of both sodium and lithium, but there were no such changes of creatinine clearance.

6. Another six normal volunteers were studied on each of two days. On each day they drank 2 l of water over 30 min and then water to replace their urinary losses. They also received loading doses and maintenance infusions of inulin and sodium para-aminohippurate. Once a full water diuresis had become established, each subject received an infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) plus carrier on one day and carrier alone on the other, exactly as before. The excretion of sodium and solute-free water, and the clearances of inulin and para-aminohippurate were recorded.

7. The infusion of atrial peptide increased the rates of excretion of both sodium and solute-free water. It also increased the clearance of inulin, but not that of para-aminohippurate.

8. These results suggest that, in our volunteers, infusion of human α-atrial natriuretic peptide increases sodium excretion mainly by increasing the delivery of

sodium along the renal tubule from sites upstream of the loop of Henle. This occurs partly as the result of an increase in the rate of glomerular filtration, but the peptide also increases the fractional excretion of filtered lithium and this suggests that human α-atrial natriuretic peptide can also reduce the fractional reabsorption of filtered sodium from the proximal tubule in man.

INTRODUCTION

Mammalian atrial myocytes contain potent natriuretic peptides that can be released into the circulation in response to the atrial stretch that occurs during expansion of the blood volume in both animals (Lang, Thölken, Ganten, Luft, Ruskoaho & Ungar, 1985) and man (Sagnella, Markandu, Shore & MacGregor, 1985). It has long been speculated that a hormone released in response to atrial stretch mediates the natriuresis caused by expansion of the extracellular fluid volume, and, recently, the circulating atrial natriuretic peptides have become attractive candidates for such a role (Balfour, 1985). This would, however, presuppose several pieces of evidence. For example, both endogenous and exogenous, synthetic, atrial natriuretic peptides should be able to reproduce at least some of the intrarenal mechanisms and urinary changes entrained by expansion of the volume of the extracellular fluid without there being any such expansion. Unfortunately, however, there is no general consensus concerning the mechanism by which exogenous atrial natriuretic peptides increase sodium excretion. These peptides are usually found to increase the filtered load of sodium in experimental animals (Maack, Camargo, Kleinert, Laragh & Atlas, 1985), but it has also been reported that a synthetic natriuretic peptide which partly corresponds to the amino acid sequence of the major endogenous atrial natriuretic peptide of the rat will increase lithium clearance more than inulin clearance in dogs (Burnett, Granger & Opgenorth, 1984). This suggests that the synthetic peptide can depress the fractional reabsorption of filtered sodium by the proximal tubule (Thomsen, 1984). However, the same synthetic peptide has no effect on sodium reabsorption from the isolated rabbit proximal tubule (Baum & Toto, 1986), and micropuncture studies in rats have instead suggested that, if natriuretic peptides have an effect in addition to increasing the rate of glomerular filtration, it is to inhibit tubular sodium reabsorption beyond the loop of Henle (Briggs, Steipe, Schubert & Schnermann, 1982; Huang, Lewicki, Johnson & Cogan, 1985). Nevertheless, these micropuncture studies have only examined the responses of superficial cortical nephrons. Moreover, the intrarenal effects of the main endogenous circulating atrial natriuretic peptide in each species may differ subtly from the effect of even close synthetic or endogenous analogues; it has been shown, for example, that the synthetic peptides, rat atriopeptin I and rat atriopeptin II, which lack seven and five of the terminal amino acids of the main circulating rat atrial natriuretic peptide respectively, have different natriuretic actions in the rat although they, themselves, differ by only two amino acid residues (Hansell & Ulfendahl, 1986). Consequently, we have examined the effects of synthetic human α-atrial natriuretic peptide on the lithium and solute-free water clearances of normal volunteers. These clearance techniques have the advantage of examining the sum of functioning nephrons in unstressed subjects (Goldberg, 1973), and human α-atrial natriuretic peptide is the major

circulating moiety in man (Yamaji, Ishibashi & Takaku, 1985). Some of these results have already been presented briefly to the Physiological Society (Brown & Corr, 1986; Brown, 1986).

METHODS

Studies were conducted with the approval of the Hammersmith Hospital Ethical Committee and with the informed consent of each volunteer.

The studies were in three groups. In each group, subjects received synthetic human (twenty-eight amino acid) α-atrial natriuretic peptide (Penninsula Laboratories, Merseyside, U.K.). Fractionation of this peptide by high-performance liquid chromatography using a Waters μ-Bondapak C-18 column (Millipore (U.K.) Ltd.) and a 20:80% to 40:60% acetonitrile to trifluoroacetic acid gradient running over 10 min revealed two major peaks in the ratio 12:1 when detected at 220 nm. These corresponded to the running positions of human α-atrial natriuretic peptide and its sulphoxidized form respectively. The synthetic atrial peptide was always infused intravenously in a carrier of 48 ml of 0·9% sodium chloride and 2 ml of human serum albumin (Evans Medical, Merseyside, U.K.), and given at a final rate of 50 ml^{-1} h^{-1}. The peptide solution was always made up to give a final, nominal rate of infusion of 15 pmol synthetic human α-atrial natriuretic peptide kg^{-1} min^{-1}. The recovery of peptide from infusate in dead space at the end of each infusion was determined to be 56 ± 4% (mean recovery $\pm$ S.E. of mean) using a radioimmunoassay described elsewhere (Anderson, Struthers, Payne, Slater & Bloom, 1986).

Group 1

Eight normal male volunteers were studied on each of two days in random order and at least one week apart. Volunteers neither ate nor drank from 21.00 h on the day before each study until 12.30 h on the day of the study. On this day, subjects voided at 09.30 h and then remained quietly seated except to void hourly urine collections until 12.30 h.

On both days between 09.30 and 12.30 h, synthetic arginine vasopressin ($5{\cdot}5 \times 10^{-7}$ i.u. kg^{-1} min^{-1}) (Pitressin, Parke-Davies, U.K.) dissolved in 0·9% sodium chloride solution was infused intravenously at 20 ml h^{-1}. On one day, between 10.30 and 11.30 h, each subject also received an intravenous infusion of synthetic human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}). On the other day between 10.30 and 11.30 h, each subject received the carrier infusion without peptide.

10 ml of venous blood was collected into lithium heparin at 10.00, 11.00 and 12.00 h.

Plasma and urine were assayed for electrolytes by flame photometry, for osmolality by depression of freezing point and for creatinine by the Jaffe method (Creatinine Analyser 2, Beckman Instruments, U.S.A.).

Blood pressure and pulse were recorded automatically (Dinamap, Critikon Inc., U.S.A.) in triplicate every 30 min from 09.45 h on each day.

The clearance of solute-free water (C_{H_2O}) was calculated as

$$C_{H_2O} = \left(1 - \frac{U_{osm}}{P_{osm}}\right) \times V,$$

where U_{osm} and P_{osm} are the simultaneous urinary and plasma osmolalities respectively, and V is the simultaneous rate of urinary volume flow. Negative values of C_{H_2O} occur when the urine is hypertonic to the plasma and, by convention, these negative values are refered to as solute-free water reabsorption (T_{H_2O}), where

$$T_{H_2O} = -C_{H_2O}.$$

Group 2

Six normal male volunteers were studied on each of two days in random order and at least one week apart. They practised the same dietary restrictions as in Group 1, except that they ingested 500 mg of lithium carbonate with 20 ml of water at 23.00 h before each study. Otherwise the study was exactly as for Group 1 except that no infusion of arginine vasopressin was made.

Collections of urine and blood were made exactly as in Group 1, except that each blood sample included a further 2 ml to be anticoagulated with potassium–EDTA for the estimation of lithium concentrations.

Plasma and urinary electrolytes, osmolality and creatinine were measured as in Group 1.

Group 3

Six normal male volunteers were studied on each of two days in random order and at least one week apart. Volunteers neither ate nor drank from 21.00 h on the day before each study until a drink of 2 l of water over 30 min starting at 08.30 h on each study day. Thereafter, subjects remained quietly seated except to void every 20 min, and, every 20 min, they drank water equivalent to their urinary volume plus 20 ml. In this way, a high, stable urine flow of more than 12 ml min^{-1} was achieved after about 2 h. At 09.00 h, each subject received an intravenous loading dose of 10 % (w/v) inulin (50 mg kg^{-1}) (Inulin Injection, B.P., T. Kerfoot & Co., Lancs, U.K.) and 20 % (w/v) sodium para-aminohippurate (8 mg kg^{-1}) (Merck Sharp & Dohme, U.S.A.). Inulin was then infused intravenously at 32·5 mg min^{-1} and sodium para-aminohippurate at 12 mg min^{-1} until 12.30 h.

Clearances were measured every 20 min, beginning with the 20 min collection of urine made once the rates of flow of three successive urinary collections were within 1 ml min^{-1} of each other. Once a basal clearance period had been recorded, synthetic human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) on one day, or its carrier alone on the other, was infused as in Group 1.

10 ml of venous blood was drawn into lithium heparin at the mid-point of each clearance period. Plasma and urinary electrolytes and osmolality were determined as before. Plasma and urinary inulin concentrations were determined by a rescorcinol method (Schriener, 1950). Plasma and urinary para-aminohippurate was estimated by a diazo-dye method (Smith, Finkelstein, Aliminosa, Crawford & Graber, 1945).

Results are presented as means and the standard error of these means. Standard deviations of regression coefficients were found by analysis of variance. The significance of comparisons of means and of regression coefficients was assessed using the t distribution.

RESULTS

Group 1

Blood pressure and pulse. The infusion of atrial peptide had a small hypotensive effect. The mean systolic pressure at the start of both the control day and the day with atrial peptide was 114 ± 2 mmHg. There was a gradual fall in the mean systolic pressures during the infusion of peptide as compared with the corresponding pressures on the control day, but this difference achieved significance only for the arterial pressures measured after the end of the infusion (Fig. 1). Thus, the mean systolic arterial pressure 15 min after the end of the infusion of atrial peptide was 111 ± 1 mmHg compared to 118 ± 2 mmHg at the same time on the control day ($0{\cdot}01 > P > 0{\cdot}001$; $t = 3{\cdot}7$; $N = 7$, where N is the number of degrees of freedom). The corresponding systolic pressures 45 min after the end of the peptide and carrier infusions were 109 ± 2 and 116 ± 3 mmHg respectively ($0{\cdot}05 > P > 0{\cdot}01$; $t = 2{\cdot}9$; $N = 7$). There were no significant differences between corresponding mean diastolic arterial pressures on the two days.

On the day of peptide infusion, the pulse rate rose from 60 ± 2 beats min^{-1} 45 min before the start of the infusion to 64 ± 2 beats min^{-1} at 45 min after the end of the infusion. There was no such rise on the control day. However, at no time was the pulse rate on the day of infusion significantly different from the corresponding pulse rate on the control day (Fig. 1).

Solute excretion. The changes that occurred in the urinary solutes on the control day and on the infusion day are summarized in Table 1. On the control day, there were no significant changes in either creatinine clearance or urinary osmolality. Since subjects were dehydrated, hourly urine flow was approximately 50–100 ml h^{-1} and, although the subjects were practised at completely voiding their bladders, errors of

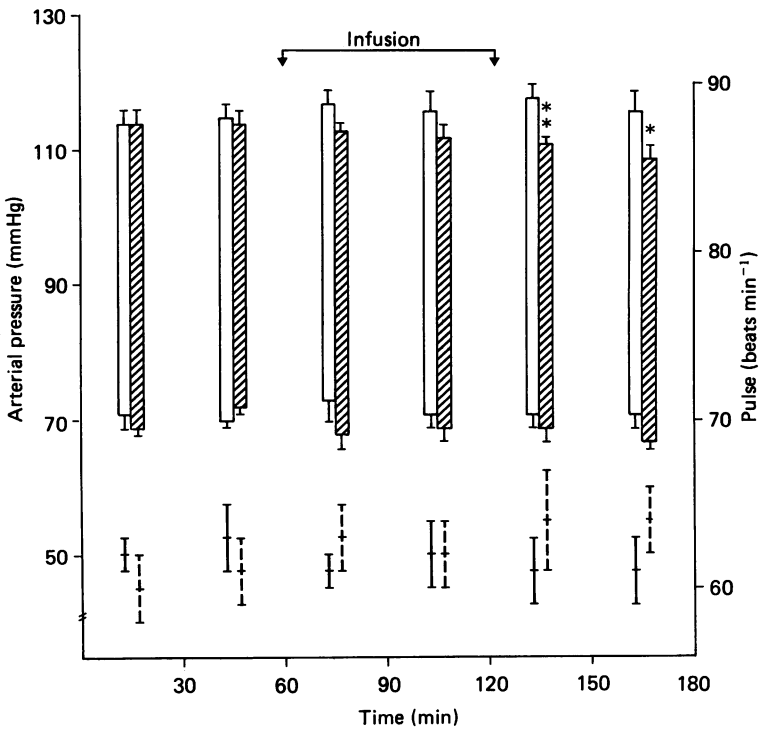

Fig. 1. Changes of arterial pressure and pulse over a period of 3 h on each of two days. Eight subjects received an infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) over the second of the 3 h on one day (hatched bars, dashed lines). On the other day, they received an infusion of carrier alone over the corresponding hour (open bars, continuous lines). Open and hatched blocks represent arterial pressure, while continuous and dashed lines represent pulse. Results expressed as means ± S.E. of mean. * = $P < 0.05$, ** = $P < 0.001$ between days.

TABLE 1. Changes in urinary parameters during three successive hourly urine collections on control day and day of peptide infusion of experiments in Group 1

		First hour	Second hour	Third hour
G.F.R. (ml min^{-1})	Control	132 ± 16	121 ± 9	118 ± 10
	Peptide	138 ± 10	145 ± 6	132 ± 7
Osmolality (mosmol kg^{-1})	Control	951 ± 36	821 ± 43	829 ± 40
	Peptide	873 ± 28	822 ± 31	871 ± 40
Sodium excretion per unit G.F.R. (μmol ml^{-1})	Control	0·82 ± 0·23	1·19 ± 0·21	1·09 ± 0·20
	Peptide	1·24 ± 0·26	2·65 ± 0·45	1·54 ± 0·30
Potassium excretion per unit G.F.R. (μmol ml^{-1})	Control	0·67 ± 0·13	0·80 ± 0·13	0·84 ± 0·11
	Peptide	0·67 ± 0·10	0·76 ± 0·10	0·63 ± 0·08

A background infusion of arginine vasopressin ($5{\cdot}5 \times 10^{-7}$ i.u. kg^{-1} min^{-1}) was given during all 3 h on both days. On the control day (upper figures in each row), carrier without atrial peptide was infused over the central hour. On the day of peptide infusion (lower figures in each row), atrial peptide was infused (15 pmol kg^{-1} min^{-1}) with the carrier over the central hour. G.F.R., glomerular filtration rate.

emptying may have been significant at these rates of urinary flow. Consequently, rates of urinary excretion are presented per unit of creatinine clearance. The rate of sodium excretion per unit of creatinine clearance increased significantly from $0{\cdot}82 \pm 0{\cdot}23$ to $1{\cdot}19 \pm 0{\cdot}21$ μmol ml^{-1} between the first and second hour of the control day ($0{\cdot}001 > P$; $t = 11$; $N = 7$), and the rate of potassium excretion per unit of creatinine clearance also rose from $0{\cdot}67 \pm 0{\cdot}13$ to $0{\cdot}80 \pm 0{\cdot}13$ μmol ml^{-1} between the first and second hours on the same day ($0{\cdot}01 > P > 0{\cdot}001$; $t = 4{\cdot}4$; $N = 7$).

The changes on the day with infusion of atrial peptide were different. The apparent creatinine clearance increased between the base-line hour and the infusion hour from

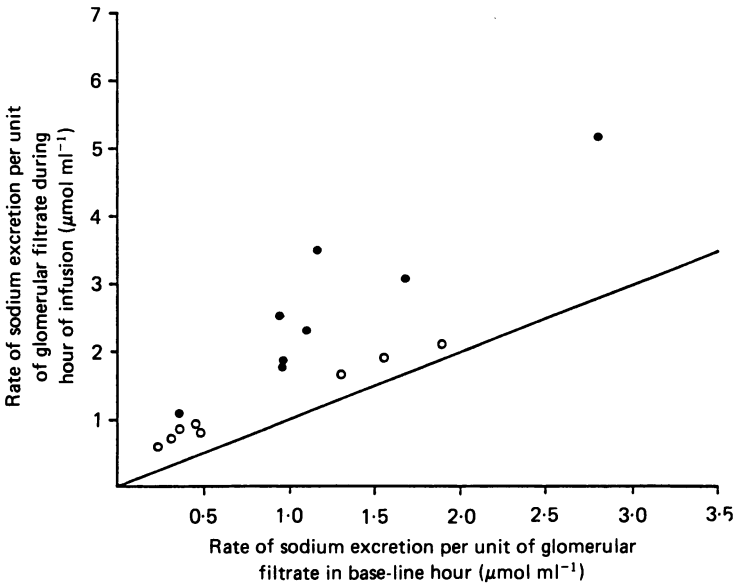

Fig. 2. Changes in sodium excretion during the infusion of atrial peptide or of carrier alone. Eight subjects were studied on each of two days. On one day, a 1 h collection of urine was made and then subjects received an infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) for 1 h, after which urine was again collected (filled circles). The other day was similar except that the carrier was infused without atrial peptide (open circles). The rate of sodium excretion per unit of creatinine clearance is compared for the basal and infusion hour on each day. The continuous line is the line of equality representing no change in sodium excretion per unit of creatinine clearance between the first and the second hour of each day.

138 ± 10 to 145 ± 6 ml min^{-1} but this was not significant ($P > 0{\cdot}1$; $t = 0{\cdot}79$; $N = 7$). Similarly, the creatinine clearance during the hour of peptide infusion on one day was not significantly different from that during infusion of carrier alone on the other (145 ± 6 *versus* 121 ± 9 ml min^{-1}; $P > 0{\cdot}1$; $t = 1{\cdot}8$; $N = 7$). There was also no significant change in urinary osmolality during the hour of peptide infusion compared either to the base-line hour on the same day (822 ± 31 *versus* 873 ± 28 mosmol kg^{-1} respectively; $P > 0{\cdot}05$; $t = 2{\cdot}0$; $N = 7$) or to the hour of carrier infusion on the control day (822 ± 31 *versus* 821 ± 43 mosmol kg^{-1}).

Infusion of atrial peptide caused the rate of sodium excretion to increase, but this increase was proportional to the rate of sodium excretion which immediately

preceded the infusion of peptide. This proportionality did not occur on the control day during the infusion of carrier alone, and these relationships are shown in Fig. 2. Thus, the coefficient for the regression of the rate of sodium excretion per unit of glomerular filtrate during the hour of peptide infusion on the same rate for the hour immediately before peptide was infused was $1{\cdot}60 \pm 0{\cdot}25$. This coefficient was significantly different to the corresponding value on the control day ($1{\cdot}60 \pm 0{\cdot}25$ *versus* $0{\cdot}91 \pm 0{\cdot}05 > P > 0{\cdot}01$; $t = 2{\cdot}6$; $N = 12$). The coefficient on the day of peptide infusion was greater than 1 ($P = 0{\cdot}06$; $t = 2{\cdot}5$; $N = 6$).

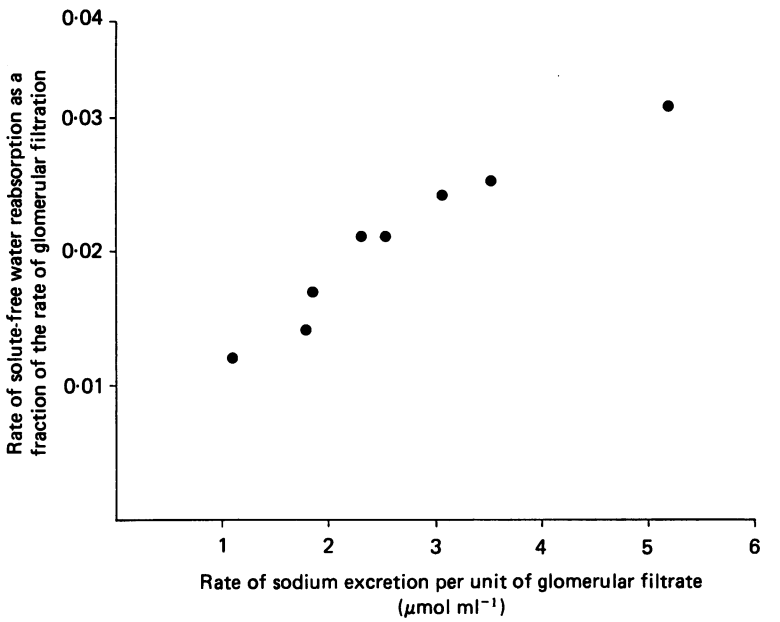

Fig. 3. Relationship between solute-free water reabsorption and sodium excretion in urinary samples collected during the infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) into eight volunteers, each for 1 h. The volunteers were dehydrated and also received a background infusion of arginine vasopressin.

Despite these changes of sodium excretion, the infusion of human α-atrial natriuretic peptide did not increase potassium excretion. The rate of potassium excretion per unit of creatinine clearance during the hour of peptide infusion was $0{\cdot}76 \pm 0{\cdot}10$ μmol ml^{-1}, whereas it was $0{\cdot}80 \pm 0{\cdot}13$ μmol ml^{-1} during the corresponding hour of infusion of carrier alone on the control day. There were no significant differences between the rates of potassium excretion per unit of creatinine clearance at any time during the day of peptide infusion when compared to the corresponding time intervals on the control day.

Solute-free water balance. On the control day, there was a small increase in the rate of solute-free water reabsorption expressed as a fraction of the creatinine clearance, from $0{\cdot}0081 \pm 0{\cdot}0009$ in the hour before carrier infusion to $0{\cdot}0098 \pm 0{\cdot}0010$ in the hour of carrier infusion ($0{\cdot}01 > P > 0{\cdot}001$; $t = 4{\cdot}6$; $N = 7$). There was, however, no significant regression of the rate of solute-free water reabsorption as a fraction of creatinine clearance during carrier infusion on the rate of sodium excretion per unit

of creatinine clearance during the hour of carrier infusion, the coefficient of regression being $4{\cdot}67 \times 10^{-3} \pm 3{\cdot}30 \times 10^{-3}$ ml μmol^{-1} ($P > 0{\cdot}1$; $t = 1{\cdot}4$; $N = 6$).

In contrast, on the day of peptide infusion, there was a greater increase in the rate of solute-free water reabsorption as a fraction of creatinine clearance, from $0{\cdot}0123 \pm 0{\cdot}0022$ in the hour before infusion to $0{\cdot}0208 \pm 0{\cdot}0021$ during the hour of infusion of atrial natriuretic peptide. In particular, there was a significant regression of the rate of solute-free water reabsorption as a fraction of creatinine clearance on

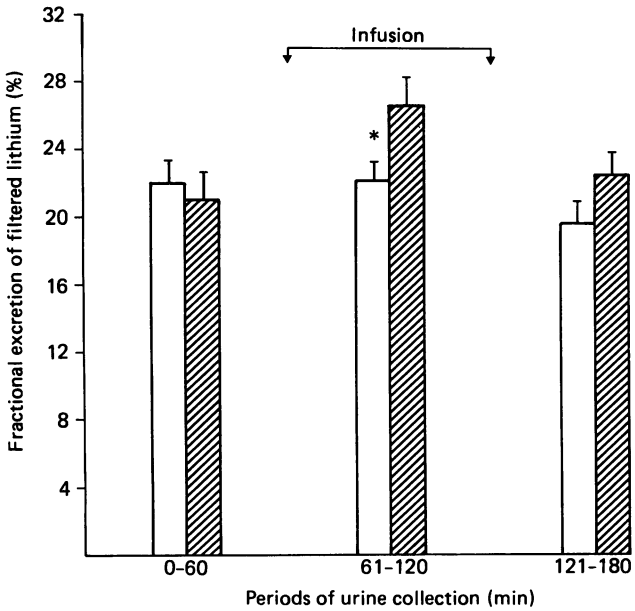

Fig. 4. Changes of the fractional excretion of filtered lithium during the infusion of atrial peptide or of carrier alone. Six subjects were studied for 3 h on each of two days after ingesting a loading dose of lithium. On one day, they received an infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) over the second of the 3 h (hatched bars). On the other day, they received an infusion of carrier alone over the corresponding hour (open bars). On each day, urinary samples were collected individually over each of the 3 h. Results expressed as means and S.E. of mean. * = $P < 0{\cdot}05$ between days.

the rate of sodium excretion per unit of creatinine clearance during the hour of atrial peptide infusion, the coefficient of regression being $4{\cdot}64 \times 10^{-3} \pm 0{\cdot}46 \times 10^{-3}$ ml μmol^{-1} ($P < 0{\cdot}001$; $t = 9{\cdot}7$; $N = 6$). This relationship is shown in Fig. 3.

Group 2

The effect of infusion of atrial natriuretic peptide on the excretion of lithium was investigated in these volunteers. The same rate of infusion of atrial peptide was used as in Group 1, and, once again, the measured creatinine clearance was not significantly affected. Thus, the creatinine clearance was 131 ± 9 ml min^{-1} during the base-line hour and 138 ± 5 ml min^{-1} during the hour of peptide infusion on the day on which atrial peptide was given ($P > 0{\cdot}1$; $t = 0{\cdot}96$; $N = 5$). Similarly, there was no significant difference between the creatinine clearance during the hour of peptide infusion on

one day and that during the hour of carrier infusion on the other (138 ± 5 *versus* 134 ± 5 ml min^{-1}; $P > 0{\cdot}1$; $t = 1{\cdot}2$; $N = 5$).

As before, the infusion of atrial peptide was natriuretic. Thus, the rate of sodium excretion per unit of creatinine clearance was initially similar on both the day of peptide infusion and on the day of infusion of carrier alone ($1{\cdot}30 \pm 0{\cdot}36$ *versus* $1{\cdot}09 \pm 0{\cdot}28$ μmol ml^{-1} respectively; $P > 0{\cdot}1$; $t = 1{\cdot}95$; $N = 5$). However, sodium excretion per unit of creatinine clearance during the hour of infusion was considerably

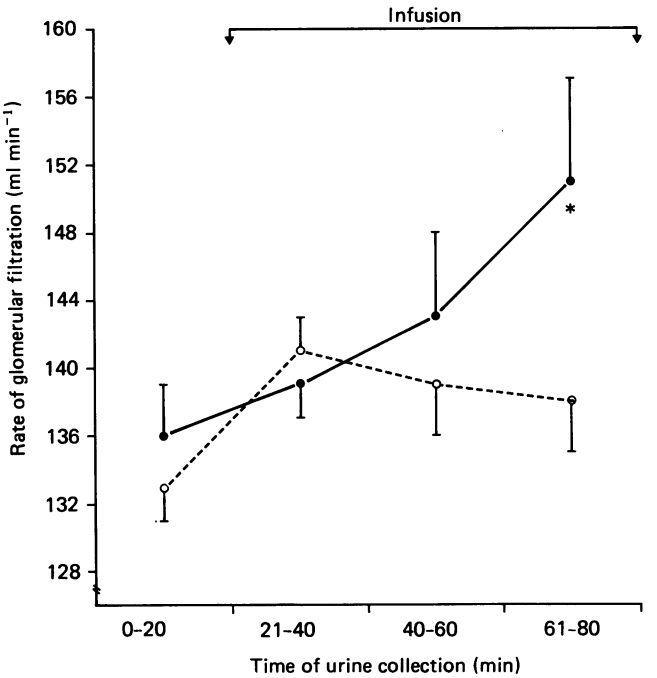

Fig. 5. Changes of glomerular filtration measured as inulin clearance during the infusion of atrial peptide or of carrier alone. Six subjects who were undergoing a maximal water diuresis were studied for four successive 20 min urinary collections on each of two days. On one day, they received an infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) for the period of the last three collections (continuous line). On the other day, they received an infusion of carrier alone for the corresponding time (dashed line). * = $P < 0{\cdot}05$ between days.

greater on the day of peptide infusion than on the day when carrier alone was infused ($2{\cdot}14 \pm 0{\cdot}50$ *versus* $1{\cdot}21 \pm 0{\cdot}32$ μmol ml^{-1} respectively; $0{\cdot}05 > P > 0{\cdot}01$; $t = 3{\cdot}61$; $N = 5$).

Infusion of atrial peptide caused a significant increase in the ratio of lithium clearance to creatinine clearance (Fig. 4). On the day of peptide infusion, this ratio increased from $21{\cdot}0 \pm 1{\cdot}7$ to $26{\cdot}6 \pm 1{\cdot}7$ % between the base-line hour and the hour of atrial peptide infusion ($0{\cdot}05 > P > 0{\cdot}01$; $t = 3{\cdot}93$; $N = 5$). Comparing the ratio for the base-line hour on the control day with that of the base-line hour of the day of peptide infusion, there was no significant difference ($22{\cdot}0 \pm 1{\cdot}4$ *versus* $21{\cdot}0 \pm 1{\cdot}7$ % respectively; $P > 0{\cdot}1$; $t = 1{\cdot}14$; $N = 5$). There was, however, a significant difference

between the ratios of lithium to creatinine clearances for the hour of carrier infusion on the control day against the hour of atrial peptide infusion on the other day ($22{\cdot}2 \pm 1{\cdot}1$ *versus* $26{\cdot}6 \pm 1{\cdot}7$ % respectively; $0{\cdot}05 > P > 0{\cdot}01$; $t = 2{\cdot}74$; $N = 5$).

Group 3

The effects of infusion of atrial natriuretic peptide on the excretion of solute-free water, inulin and para-aminohippurate were examined in this group of hydrated

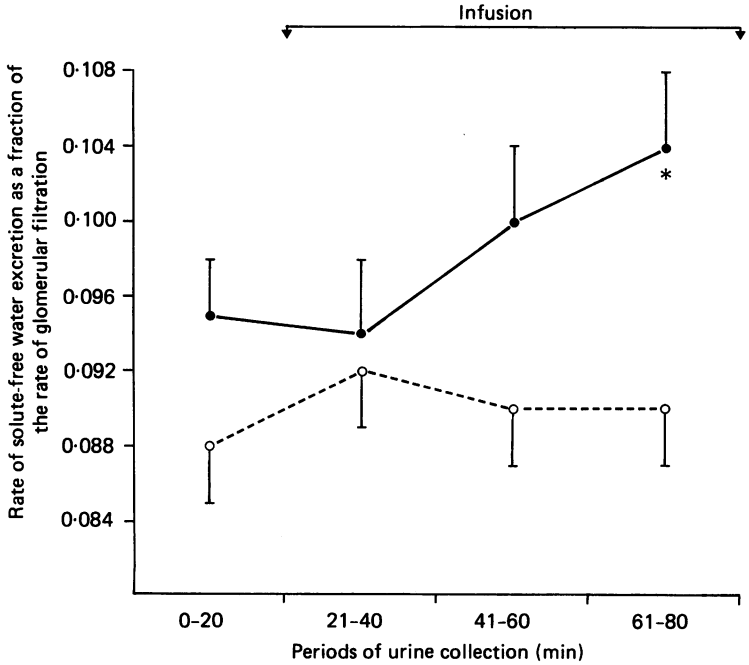

Fig. 6. Changes of solute-free water excretion as a fraction of inulin clearance during the infusion of atrial peptide or of carrier alone. Six subjects who were undergoing a maximal water diuresis were studied for four successive 20 min urinary collections on each of two days. On one day they received an infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) for the period of the last three collections (continuous line). On the other day, they received an infusion of carrier alone for the corresponding time (dashed line). Results are expressed as mean and S.E of mean. * = $P < 0{\cdot}05$ between days.

volunteers who were undergoing a maximal water diuresis. The same 1 h infusion of peptide was used as in Groups 1 and 2.

On the day on which carrier alone was infused, there were no significant changes in the rate of glomerular filtration calculated as the inulin clearance (Fig. 5). On the day when atrial peptide was delivered with the carrier, the initial rate of glomerular filtration was not significantly different to that on the control day (136 ± 3 *versus* 133 ± 2 ml min^{-1} respectively; $P > 0{\cdot}1$; $t = 1{\cdot}00$; $N = 5$). However, the rate of glomerular filtration rose gradually during the infusion of atrial peptide and, by the end of the infusion, reached 151 ± 6 ml min^{-1}, which was significantly different to the level of 138 ± 3 ml min^{-1} at the end of the infusion of carrier on the control day ($0{\cdot}05 > P > 0{\cdot}01$; $t = 2{\cdot}69$; $N = 5$).

The rate of sodium excretion per unit of glomerular filtrate increased from $0{\cdot}96 \pm 0{\cdot}14$ μmol ml^{-1} for the urine collected for 20 min before the start of the infusion of peptide to $3{\cdot}41 \pm 0{\cdot}85$ μmol ml^{-1} for the urine collected in the last 20 min of the infusion of atrial peptide. On the day of the infusion of carrier alone, the corresponding rates were $0{\cdot}92 \pm 0{\cdot}22$ and $0{\cdot}94 \pm 0{\cdot}22$ μmol ml^{-1} respectively. Associated with this, the infusion of atrial natriuretic peptide caused a sharp increase in the clearance of solute-free water (Fig. 6). Thus, the clearance of solute-free water as a fraction of the

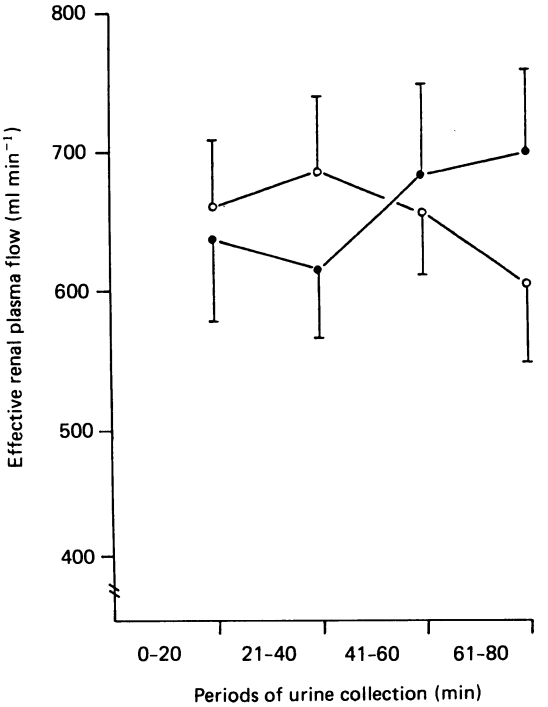

Fig. 7. Changes of effective renal plasma flow during the infusion of atrial peptide or of carrier alone. Six subjects who were undergoing a maximal water diuresis were studied for four successive 20 min urinary collections on each of two days. On one day they received an infusion of human α-atrial natriuretic peptide (15 pmol kg^{-1} min^{-1}) for the period of the last three collections (filled circles). On the other day, they received an infusion of carrier alone for the corresponding time (open circles). The clearance of para-aminohippurate was measured for each urinary collection. Results expressed as means and S.E. of mean.

clearance of inulin for urine collected for 20 min before the start of the peptide infusion was $0{\cdot}095 \pm 0{\cdot}003$ and this fraction increased to $0{\cdot}104 \pm 0{\cdot}004$ for urine collected during the final 20 min of the infusion. On the control day, the corresponding values were $0{\cdot}088 \pm 0{\cdot}003$ and $0{\cdot}090 \pm 0{\cdot}003$ respectively. There was a significant difference between values for the final clearance period on each day ($0{\cdot}104 \pm 0{\cdot}004$ *versus* $0{\cdot}090 \pm 0{\cdot}003$; $0{\cdot}05 > P > 0{\cdot}01$; $t = 3{\cdot}3$; $N = 5$).

Finally, there were no consistent changes of the effective renal plasma flow as measured by the clearance of para-aminohippurate between the control day and the day of peptide infusion (Fig. 7).

DISCUSSION

An important conclusion of this study is that infusion of synthetic human α-atrial natriuretic peptide increases the rate of reabsorption of solute-free water in dehydrated volunteers and increases the rate of excretion of solute-free water in hydrated volunteers. These changes of solute-free water balance occur at a time when the infusion of atrial peptide has also caused an increase in sodium excretion. Furthermore, the rate of solute-free water reabsorption in dehydrated subjects during the action of atrial peptide is closely proportional to the associated natriuresis (Fig. 3). These changes imply that human α-atrial natriuretic peptide can exert its main effect on sodium excretion by increasing the rate at which sodium leaves the proximal tubule to enter the loop of Henle and, subsequently, the urine.

Solute-free water is thought to be generated by the active reabsorption of luminal sodium chloride across the water-impermeable epithelium of the ascending limb of the loop of Henle. Therefore, the rate of excretion of solute-free water in hydration and dehydration provides an estimate of the activity of the ascending limb of the loop of Henle (Goldberg, 1973). However, a number of precautions are needed before the changes in solute-free water clearance induced by atrial natriuretic peptide can be interpreted in this way. The release of antidiuretic hormone which is induced by dehydration in dogs can be prevented by large doses of synthetic rat atrial natriuretic peptide (Samson, 1985). There is also evidence that rat atrial natriuretic peptide can inhibit the change of hydraulic conductivity which is caused by antidiuretic hormone in isolated perfused rabbit collecting ducts (Dillingham & Anderson, 1986). Any change in the permeability of the collecting duct to water during the action of atrial peptides would complicate the use of solute-free water clearance as a measure of the activity of the loop of Henle. Consequently, our dehydrated subjects in Group 1 received a supplementary intravenous infusion of antidiuretic hormone (arginine vasopressin) to prevent any decrease in urinary osmolality. The use of solute-free water reabsorption as a measure of the activity of the loop of Henle also requires that the tubular fluid has achieved isotonicity by the end of the cortical collecting duct (Goldberg, 1973). The measured rate of solute-free water reabsorption will underestimate the rate at which water is reabsorbed from the medullary collecting duct if hypotonic tubular fluid leaves the cortical collecting duct. This is known to happen at the exaggerated rates of tubular flow which occur during profound osmotic diuresis in the dog because a hypotonic urine is excreted despite maximum levels of antidiuretic hormone (Orloff, Wagner & Davidson, 1958). However, in dehydrated man, urine does not become hypotonic, even during extreme osmotic diuresis with rates of urine flow approaching 20% of the rate of glomerular filtration (Goldberg, McCurdy & Ramirez, 1965). The dose of human α-atrial natriuretic peptide used here was selected to give much lower rates of urinary flow during dehydration. These rates of flow were always less than 2% of the rate of glomerular filtration, the usual range of urinary flow for dehydrated subjects on normal Western diets (Boyarsky & Smith, 1957). Without these precautions, changes in solute-free water balance become too complex to anticipate, and this may explain the apparent conflict of the present results with both of the previous studies in man, which have shown that exogenous human α-atrial natriuretic peptide decreases rather than increases the rate of

solute-free water reabsorption in dehydrated volunteers (Kuribayashi, Nakazato, Tanaka, Nagamine, Kurihara, Kangawa & Matsuo, 1985; Weidmann, Hasler, Gnädinger, Lang, Uehlinger, Shaw, Rascher & Reubi, 1986). With these precautions, the correspondence of the directional changes of solute-free water clearance in both dehydration and hydration, and the proportional relationship between solute-free water reabsorption and sodium excretion which we have shown strongly suggest that human α-atrial natriuretic peptide can increase sodium excretion in man principally by actions on segments of the nephron which are upstream of the loop of Henle.

The effects of human α-atrial natriuretic peptide on the clearances of inulin and lithium corroborate proximal sites of action in man. The infusions of human α-atrial natriuretic peptide given to the hydrated volunteers of Group 3 caused an increase of about 11 % in the rate of glomerular filtration. Two other groups have described the effect of exogenous human α-atrial natriuretic peptide on the rate of glomerular filtration in man. Kuribayashi *et al.* (1985) found creatinine clearance to be increased by over 200 %. However, these increases were measured at a time when the urinary minute volume was rising very sharply after a bolus injection of peptide, and, therefore, they probably represent effects introduced by the urinary dead space (Bojesen, 1954). Weidmann *et al.* (1986) catheterized and washed the bladders of their volunteers so that errors of dead space would be minimized; they found an increase in the clearance of ^{51}Cr–EDTA of about 15 %, which is comparable to the increase of inulin clearance measured here. Differences in plasma concentrations of atrial peptide may account for some differences between studies. Weidmann *et al.* (1986) gave bolus intravenous injections of 50 μg of human α-atrial natriuretic peptide followed by maintenance infusions of the peptide, and found a single plasma concentration of approximately 200 pmol l^{-1} during the maintenance infusion. It is known, however, that the plasma concentration of immunoreactive human α-atrial natriuretic peptide reaches levels of approximately 2000 pmol l^{-1} for a short time after bolus intravenous injections of 50 μg of the exogenous peptide in man (Kuribayashi *et al.* 1985). The method of infusion of atrial peptide used in the present study has already been found by others to achieve a steady plasma concentration of immunoreactive human α-atrial natriuretic peptide of approximately 300 pmol l^{-1} within 30 min of the start of the infusion (Anderson *et al.* 1986).

The increase in the filtered load of sodium caused by the 11 % increase of glomerular filtration found here could fully account for the associated increase in the rate of sodium excretion, which was, on average, only about 20 % of the increase in the filtered load of sodium. However, micropuncture, occlusion time and clearance studies suggest that lithium is a marker of proximal tubular sodium reabsorption (Hayslett & Kashgarian, 1979; Thomsen, Holstein-Rathlow & Leyssac, 1981; Thomsen, 1984). Consequently, the increased excretion of filtered lithium found in the subjects of Group 2 implies that human α-atrial natriuretic peptide can depress the fractional reabsorption of filtered sodium from the proximal tubule. A similar effect on lithium excretion has already been found by infusing dogs with a synthetic peptide which mimics part of the amino acid sequence of the main circulating atrial natriuretic peptide of rats (Burnett *et al.* 1984). Care is needed to interpret these results of lithium excretion because there is evidence that lithium can be reabsorbed by the distal tubules of severely sodium-depleted rats (Thomsen, 1977). However,

our subjects were taking diets unrestricted in their sodium content. Moreover, the same synthetic analogue of rat atrial natriuretic peptide which increased the excretion of filtered lithium in the dog also increased the excretion of filtered phosphate, again suggesting a depression of proximal tubular sodium reabsorption (Burnett *et al.* 1984). Recently, it has been reported that infusions of human α-atrial natriuretic peptide cause a pronounced phosphaturia in man (Weidmann *et al.* 1986). In fact, this study shows a substantial increase in the fractional excretion of filtered phosphate and provides a qualitative confirmation that the changes of lithium excretion described here do indeed reflect a reduction in the fractional reabsorption of filtered sodium by the proximal tubule during infusions of human α-atrial natriuretic peptide in man.

The increase of glomerular filtration caused by human α-atrial natriuretic peptide is consistent with the increase of filtration that atrial natriuretic peptides usually cause in isolated perfused kidneys and in intact animals (Maack *et al.* 1985). It is also consistent with the demonstration of specific binding of radiolabelled atrial peptides to the glomeruli of rats (Bianchi, Gutkowska, Garcia, Thibault, Genest & Cantin, 1985; Koseki, Hayashi, Torikai, Furuya, Ohnuma & Imai, 1986). On the other hand, the conclusion that human α-atrial natriuretic peptide causes natriuresis partly by an effect on the proximal tubule differs from the conclusion generally derived from animal work. In relatively dehydrated animals, without supplements of exogenous antidiuretic hormone, atrial natriuretic peptides cause urinary osmolality to fall during the natriuresis. This has led to the idea that atrial natriuretic peptides, which also have vasodilator properties, cause sodium excretion to increase by inducing vascular 'wash-out' of medullary interstitial solute (Maack *et al.* 1985). Such an idea would be consistent with the failure to demonstrate a specific reduction in the fractional reabsorption of filtered sodium from proximal tubules of superficial nephrons that have been micropunctured during the infusion of atrial peptides in rats (Briggs *et al.* 1982; Huang *et al.* 1985). Instead, these micropuncture investigations have suggested that atrial peptides cause natriuresis by depressing the fractional reabsorption of filtered sodium by tubular segments beyond the loop of Henle. In addition, microcatheterization has suggested that atrial peptides can depress sodium reabsorption from medullary collecting ducts (Sonnenberg, Honrath, Chong & Wilson, 1986). However, a fall of urinary osmolality may simply reflect an ability of atrial peptides to depress the concentration or effectiveness of circulating antidiuretic hormone, and, recently, it has been reported that the level of natriuresis caused by standard infusions of human α-atrial natriuretic peptide given to dehydrated volunteers is the same whether or not urinary osmolality is maintained in its high, dehydrated range by exogenous antidiuretic hormone (Brown & Dollery, 1986).

So far, however, radioligand studies have not reported specific binding of atrial natriuretic peptides to the epithelium of the proximal tubule (Bianchi *et al.* 1985; Koseki *et al.* 1986). Moreover, atrial peptides do not seem to affect net sodium transport by isolated perfused proximal tubules (Baum & Toto, 1986). In this regard, it may be significant that sodium transport is not altered by atrial peptide added *in vitro* to proximal tubular membrane vesicles but is altered if the rats are pre-treated with atrial peptide *in vivo* before the vesicles are prepared (Hammond, Yusufi, Knox

& Dousa, 1985). Therefore, the mechanism whereby atrial peptide affects proximal-tubular sodium reabsorption may be indirect. One possibility has already been suggested (Balfour, 1985); angiotensin II may augment reabsorption from the proximal tubule, and atrial peptides might depress this reabsorption of sodium by their effect of inhibiting the release of renin.

In conclusion, infusion of human α-atrial natriuretic peptide in man can cause increases in the filtered load of sodium and decreases in the fractional reabsorption of sodium from the proximal tubule. The association of these changes with the changes of solute-free water clearance which we describe suggest that they are responsible for the natriuretic effect of this atrial peptide in man. Similar changes are seen with the natriuresis caused by infusion of saline (Goldberg *et al.* 1965; Thomsen, 1984). To this extent, it remains possible that endogenous human α-atrial natriuretic peptide may act as a natriuretic hormone released in response to expansion of the extracellular fluid volume in man.

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