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INFLUENCE OF VARIATIONS IN HYDRATION AND IN SOLUTE EXCRETION ON THE EFFECTS OF LYSINE-VASOPRESSIN INFUSION ON URINARY AND RENAL TISSUE COMPOSITION IN THE CONSCIOUS RAT

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SUMMARY

1. The changes in urinary and renal tissue composition induced by continuous, intravenous infusion of lysine-vasopressin (60 μ-u./min. 100 g body wt. until steady-state conditions prevailed) in normally hydrated, hydropaenic, saline-loaded (0·9 %, w/v) and mannitol-loaded (15 %, w/v) rats were determined and compared with those induced in water-loaded rats.

2. Previous reports that the urinary responses to antidiuretic hormones vary both with hydration status and with concurrent solute excretion rate were confirmed.

3. The data show that variations in urinary responses were accompanied by differences in the papillary responses to lysine-vasopressin.

4. The results are discussed in terms of the effects of hydration and concurrent solute excretion on factors influencing (*a*) medullary accumulation of water and solute, (*b*) osmotic water reabsorption and (*c*) osmotic equilibration across the collecting duct; and of the effects of lysine-vasopressin on these factors.

5. It is concluded that the effects of hydration and solute excretion on the antidiuretic responses to lysine-vasopressin may be interpreted by differences in (*a*) the medullary composition prevailing at the start and (*b*) any further changes in medullary composition that can be induced under the experimental circumstances.

INTRODUCTION

The ability of the mammalian kidney to elaborate concentrated urine is influenced by the antecedent and concurrent hydration status and by the concurrent rate of solute excretion.

Prolonged overhydration reduces the urinary concentrating response to neurohypophysial antidiuretic hormone (ADH) in man (de Wardener & Herxheimer, 1957; Epstein, Kleeman & Hendrikx, 1957). Conversely, administration of ADH to hydropaenic subjects may cause little increase in urinary osmolality (Raisz, McNeely & Saxon, 1958); and in both normally hydrated man (West, Traeger & Kaplan, 1955; Jones & de Wardener, 1956; Epstein *et al.* 1957) and dog (West *et al.* 1955) even large doses of ADH do not produce as concentrated an urine as does prolonged dehydration. Various explanations of this influence of hydration have been proposed. It is generally considered that differences in glomerular filtration rate do not play a major role (West *et al.* 1955; Epstein *et al.* 1957; de Wardener & Herxheimer, 1957; Levinsky, Davidson & Berliner, 1959). A more favoured suggestion is that the tubular responsiveness to ADH is changed, e.g. through variable hydration of the tissues, including the nephron (Epstein *et al.* 1957; Levinsky *et al.* 1959); or secondary to changes in adrenocortical activity (West *et al.* 1955; Levinsky *et al.* 1959).

The influence of solute excretion rate on renal concentrating ability is shown by the observation that during osmotic diuresis induced by administration of a variety of solutes (including mannitol and various sodium salts), the osmolality of urine falls towards that of plasma even in severe hydropaenia and in the presence of supramaximal amounts of ADH; in this respect, the rat is similar to other species (Corcoran, del Greco & Masson, 1956; Koike & Kellogg, 1957). Again, various explanations of the impaired concentrating ability have been proposed (see Robinson, 1954). Most of these relate to effects on the concentrating site of changes in the volume and composition of intraluminal fluid delivered from more proximal segments of the nephron. A recent review (Berliner & Bennett, 1967) covers the pertinent literature and discusses the consequent restraints on water reabsorption by the collecting duct. However, other factors must also be involved, since during saline loading in the dog impairment of concentrating ability may be manifest even where experimental conditions are so arranged as to minimize changes in urinary flow or solute excretion (Earley & Friedler, 1964; McDonald & de Wardener, 1965). McDonald & de Wardener (1965) suggested that the presence of some circulating substance, other than vasopressin, decreased the water permeability of the distal tubule and collecting duct.

In most discussions of the influence of hydration status and of solute excretion on renal concentrating ability, little consideration has been given to a more obvious possibility. The rate of water reabsorption from the nephron is influenced not only by membrane permeability to water, but also by the osmotic reabsorptive force; the final abstraction of water from the collecting duct thus depends on medullary osmolality, as well as on

collecting duct permeability to water. We have described, previously, the profound reduction in medullary solute concentrations that occurs in the rat during water (Atherton, Hai & Thomas, 1968*b*), hypertonic mannitol (Atherton *et al.* 1968*a*) and isotonic saline (Atherton, Green & Thomas, 1970*a*) loading. It might be expected, therefore, that the urinary response to ADH would be influenced by the medullary osmolality existing at the time of ADH administration and by the maximal medullary osmolality capable of being generated under the prevailing experimental conditions.

The present investigation was performed in order to examine the extent to which the influence of hydration status and solute excretion on the urinary responses to ADH might be explained by differences in renal medullary responses. For this purpose, lysine-vasopressin was administered to normally hydrated and hydropaenic rats, and to rats infused with hypertonic mannitol and isotonic saline solutions. The changes in urinary and renal tissue composition are compared with those induced in water-loaded rats; the latter changes were described in the preceding paper (Atherton, Green & Thomas, 1971). Preliminary accounts of some of these results have been given previously (Atherton, Evans, Green & Thomas, 1970; Atherton, Green & Thomas, 1970*b*).

METHODS

Experiments were performed on five groups of conscious male albino rats (Wistar strain, weighing 218–365 g), previously maintained on a rat cake diet (21·7 % protein). In one group (hydropaenic), water was withheld for 48 hr; the others were allowed water *ad lib.* before experiments

Within each group some rats were used as controls; in others, lysine-vasopressin (LVP) was infused intravenously, at a time when previous and current data showed that an essentially steady state existed in respect to urinary flow and urinary and renal tissue composition. The dose of LVP (60 μ-u./min. 100 g body wt.) was that shown to produce near-maximal effects in water-loaded rats (Atherton *et al.* 1971); and LVP infusion was continued until essentially steady-state conditions prevailed in respect to urinary flow and osmolality. In two groups (normally hydrated and hydropaenic rats), LVP was infused in 0·9 % (w/v) isotonic saline at 0·6 μl./min. 100 g body wt. (so that the fluid load was only 0·216 ml./100 g body wt. over the 6 hr of infusion). In the other three groups, LVP was added, at appropriate times, to the fluids being infused. The experiments performed were:

Group I, normally hydrated: I*a*, controls (n = 7); I*b* LVP infusion for 6 hr (n = 5).

Group II, hydropaenic: II*a*, controls (n = 5); II*b*, LVP infusion for 6 hr (n = 5).

Group III, water loading; III*a*; controls, 7½ hr (n = 5); III*b*, LVP infusion commenced 3 hr from start of water loading and continued for 4½ hr (n = 7). These are the experiments designated Groups I*d* and V*c* respectively, in the previous paper (Atherton *et al.* 1971).

Group IV, saline loading: controls, IV*a*, 7 hr (n = 5); IV*b*, LVP infusion commenced at 3 hr from the start of saline loading and continued for 4 hr (n = 5).

Group V, mannitol loading: V*a*, controls, 6 hr (n = 5); V*b*, LVP infusion commenced at 2 hr from the start of mannitol infusion and continued for 4 hr (n = 5).

Subsequently, the start of LVP infusion and the corresponding times in the appropriate control experiments will be designated 0 hr.

More detailed descriptions of the protocol in fluid infusion experiments have been given previously: water loading by administration of hypotonic, 2·5 % dextrose to 4 % body wt. over 2 hr, with subsequent maintenance of a constant water load (Atherton *et al.* 1971); saline (0·9 %, w/v) at 0·2 ml./min (Atherton *et al.* 1970*a*); and mannitol (15 %, w/v) at 0·1 ml./min (Atherton *et al.* 1968*a*).

In the fluid-loading groups, urine samples were collected every ½ hr. In experiments where very low rates of urine flow were expected, longer collection periods (2 hr in normally hydrated, and 3 hr in hydropaenic, rats) were used in order to increase the completeness of bladder emptying.

Analyses of urine, plasma and tissue samples; calculations of tissue osmolality, tissue water and solute contents and plasma clearances; and statistical analyses were all performed as described in the preceding paper (Atherton *et al.* 1971).

RESULTS

Data concerning plasma osmolality and solute concentrations are presented in Table 1. Plasma osmolality was significantly lower in water-loaded than in the normal and hydropaenic rats. In the mannitol-infused group, the far higher plasma osmolality is attributable to the presence of osmotically active mannitol, and to the progressively increasing negative water balance (Atherton *et al.* 1968*a*).

In all five groups of rats, plasma osmolality tended to be lower in LVP-infused than in control animals; however, the differences were significant only where appreciable natriureses were associated with significant reductions in plasma Na concentration (water-loaded and saline-loaded groups) or where water retention occurred.

Plasma urea concentrations were lowest in the diuretic groups, where the high urine flows were associated with the highest urinary urea outputs and urea clearances (Fig. 3). LVP infusion caused no significant change in any group.

Urinary data concerning the normally hydrated and hydropaenic rats are presented in Fig. 1; and data for the mannitol- and saline-infused rats in Fig. 2. Within each group, initial values for the control and LVP-infused subgroups were closely similar in almost all respects, except in the normally hydrated rats where flows were lower and osmolalities slightly higher in the control rats. In the normally hydrated rats, LVP infusion caused a fall in urinary flow and an increase in osmolality (to over 2000 μ-osmole/g. H_2O) in the first 2 hr; subsequently, both remained stable. In the hydropaenic rats little change in urinary osmolality occurred throughout LVP infusion; a small, but statistically significant ($P < 0{\cdot}01$) increase in flow occurred, so that urinary osmolal output also increased, with a tendency to an increase in the outputs of individual solutes. Increased

outputs of most solutes (except K) also occurred during LVP infusion in the normally hydrated group.

In the saline-infused rats, LVP administration caused a decrease in urinary flow and an increase in osmolality (Fig. 2), but these changes were much smaller than those which occurred in the water-loaded group (Fig. 3). A substantial, but transient, natriuresis occurred in the saline-loaded group (cf. effect of LVP in water-loaded rats: see Fig. 1, Atherton *et al.* 1971). In marked contrast, administration of LVP to mannitol-infused rats caused no significant changes in urinary flow or in solute outputs (Fig. 2). In order to facilitate comparison between the effects of LVP

TABLE 1. Mean ($\pm$ S.E.) plasma osmolal, Na and urea concentrations in water-loaded, normally hydrated, hydropaenic, saline-loaded and mannitol-loaded rats; both in control experiments and after lysine-vasopressin (LVP) infusion at 60 μ-u./min. 100 g body wt. Each P value is the probability that the difference between means arose by chance (only values less than 0·05 are included)

	Osmolal (μ-osmole/g H_2O)	Urea (μ-mole/ml.)	Na (μ-equiv/ml.)
Water-loaded control (n = 4)	308 ± 3	2·70 ± 0·20	143 ± 3
LVP (n = 7)	280 ± 4	3·43 ± 0·36	132 ± 2
	< 0·001		< 0·01
Normal control (n = 6)	323 ± 2	6·20 ± 0·20	149 ± 7
LVP (n = 6)	307 ± 2	5·52 ± 0·40	145 ± 2
	< 0·001		
Hydropaenia control (n = 4)	323 ± 2	7·44 ± 0·52	146 ± 4
LVP (n = 5)	312 ± 6	7·16 ± 0·60	148 ± 3
Saline-loaded control (n = 5)	316 ± 2	2·34 ± 0·26	150 ± 3
LVP (n = 5)	285 ± 3	2·72 ± 0·18	136 ± 1
	< 0·001		< 0·01
Mannitol-loaded control (n = 5)	377 ± 8	4·64 ± 0·88	140 ± 4
LVP (n = 5)	367 ± 6	3·76 ± 0·46	140 ± 2

infusion in the various groups, data relating to the final, essentially steady-state, conditions are summarized in Fig. 3. It is evident that in respect of urinary flow and the urine/plasma concentration ratios for osmoles (U/P_{osm}) and urea (U/P_{urea}) the effects of LVP infusion differed between the groups. The effects may be further subdivided according to the concurrent rate of solute excretion or osmolal clearance (C_{osm}). In the three groups of rats with low osmolal clearances—the water-loaded, normally hydrated and hydropaenic rats—the lower the control values for U/P

osmolal and U/P urea concentration ratios, the greater were the increases induced by LVP: i.e. the antidiuretic response was greatest in the water-loaded and least in the hydropaenic group. Similarly, the lower (or more

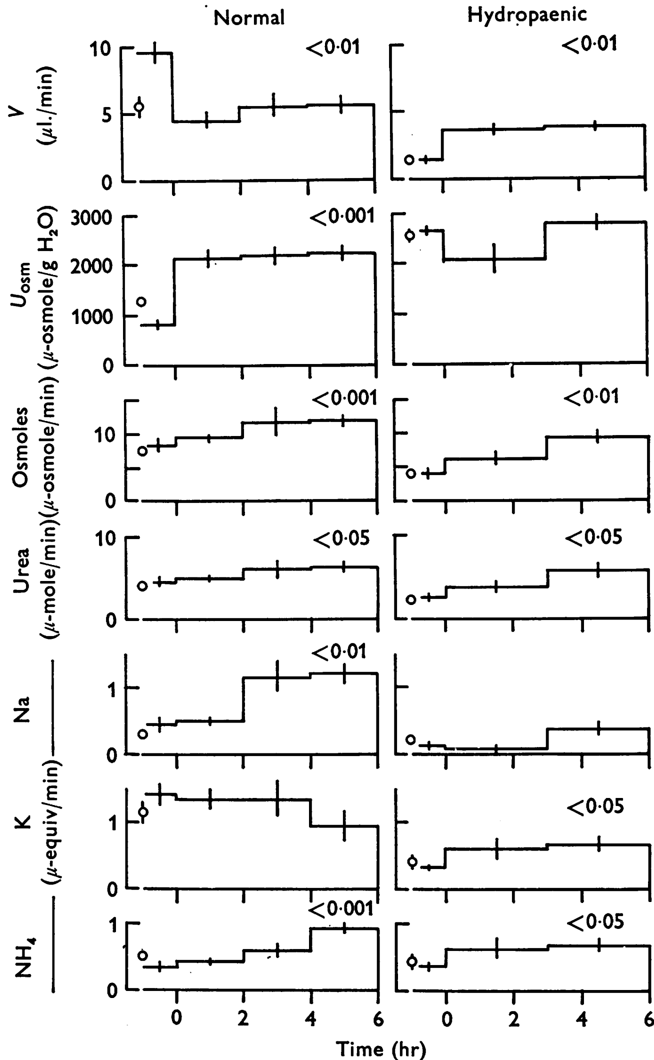

Fig. 1. Mean (± S.E.) urinary flow (V) and osmolality (U_{osm}); and urinary outputs of osmoles, urea, sodium, potassium and ammonium during continuous intravenous infusion of lysine-vasopressin (60 μ-u./min. 100 g body wt.): *left*, normally hydrated rats and *right*, hydropaenic rats. For comparison, values in the control experiments (○) are included. P values represent the probability of the difference between initial (before LVP infusion) and final values being zero; only values for $P < 0{\cdot}05$ are presented.

negative) the free-water clearance (C_{H_2O}) in the control group, the smaller was the difference between control and LVP-infused rats (Fig. 3).

The high rates of solute excretion in the mannitol, and saline, infused groups were associated with marked reductions in the responses to LVP

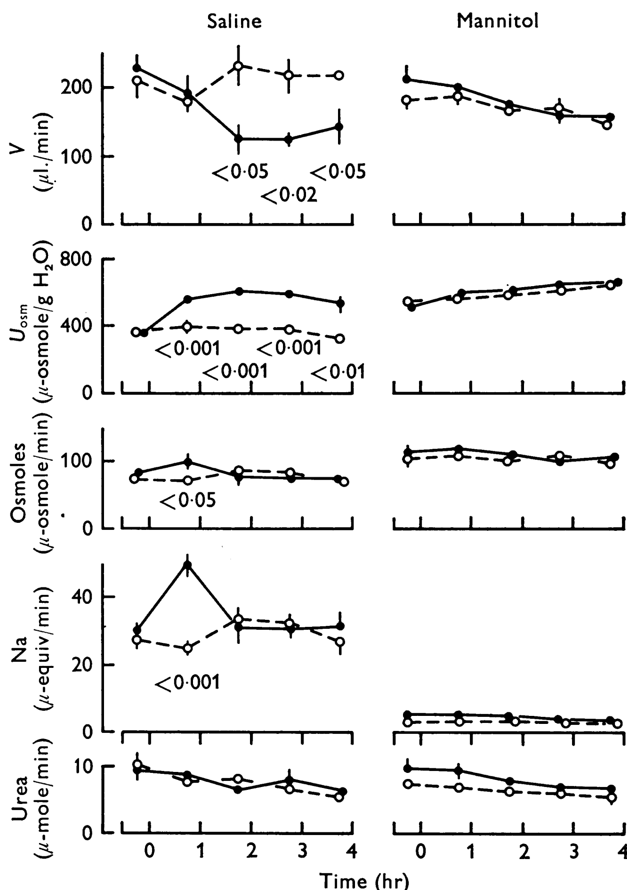

Fig. 2. Mean (± S.E.) urinary flow (V) and osmolality (U_{osm}); and urinary outputs of osmoles, sodium and urea during *left*, 0·9 % (w/v) saline infusion and *right*, 15 % (w/v) mannitol infusion: control experiments (○ - - ○); experiments with lysine-vasopressin infusion, 60 µ-u./min. 100 g body wt. (●—●). P values represent the probability of the differences between control and LVP experiments being zero; only values for $P < 0{\cdot}05$ are presented.

infusion despite the fact that urinary flows in these animals approached the high values observed in water-loaded rats; furthermore, the extent of this influence depended on the particular solute infused. Thus, in contrast to the considerable antidiuretic effect of LVP in the water-loaded rats, the

reduction in urinary flow and the increases in U/P osmolal and urea concentration ratios were much smaller in the saline-loaded group; and no significant effect occurred in the mannitol-infused rats (Fig. 3). As in the variously hydrated animals with low rates of solute excretion, free-water

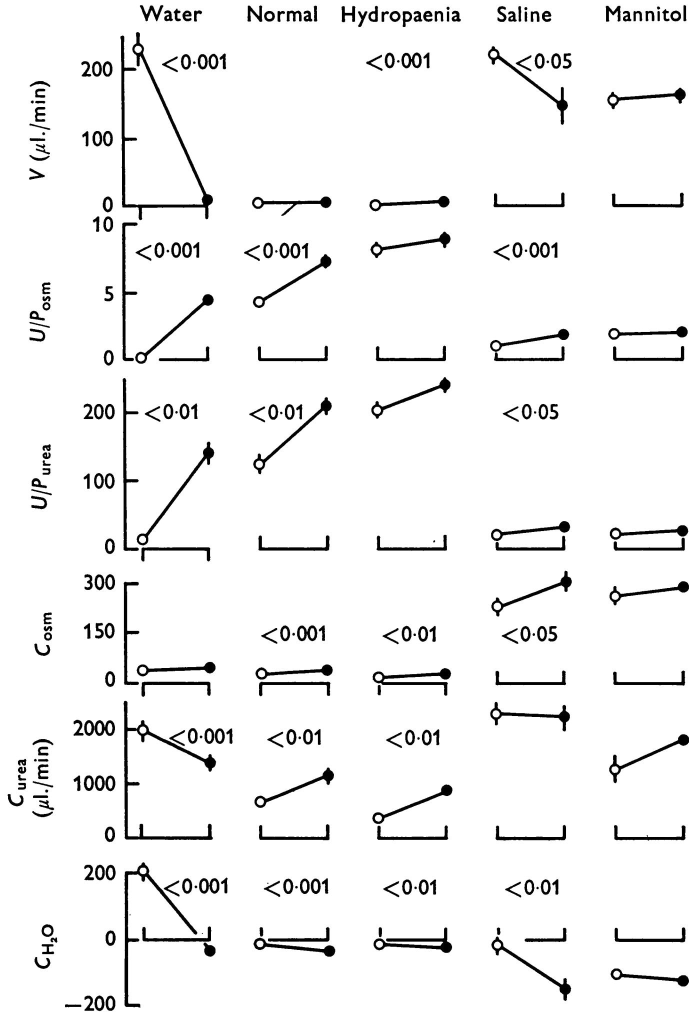

Fig. 3. Mean ($\pm$ S.E.) urinary flow (V); urine/plasma osmolal (U/P_{osm}) and urea (U/P_{urea}) concentration ratios; plasma clearances of osmoles (C_{osm}), urea (C_{urea}) and free-water (C_{H_2O}), in water-loaded, normally hydrated, hydropaenic, saline-loaded and mannitol-loaded rats. Values represent data from final periods in control experiments (○) and in experiments with lysine-vasopressin infusion, 60 μ-u./min. 100 g body wt. (●) P values represent the probability of the difference between control and LVP experiments being zero; only values for $P < 0.05$ are included.

clearances in LVP-infused, solute-loaded rats were lower (more negative) than those in the corresponding control animals; but this was substantial, and significant, only in the saline-loaded group (Fig. 3).

Osmolal clearances (C_{osm}) tended to be higher in all the LVP-infused rats than in the corresponding control animals, though this effect was not significant in the water-loaded and mannitol-infused rats. Differences in urea clearances (C_{urea}) between control and LVP-infused rats were variable in magnitude and direction.

Data concerning the influence of hydration status (Hai & Thomas, 1969), mannitol loading (Atherton *et al.* 1968*a*) and saline loading (Atherton *et al.* 1970*a*) on renal tissue composition in rats were presented and discussed previously. For this reason and because papillary tip data are qualitatively representative of those in other medullary segments, only papillary tip values are presented in Figs. 4 (solute concentrations) and 5 (water and solute contents). As with the urinary data, the differences between control and LVP-infused rats may be separated, for convenience, into those observed at low and high rates of solute excretion.

In the three groups with low rates of solute output the differences in papillary solute concentrations between control and LVP-infused rats varied with the concentrations in the control animals, which, in turn, varied with the hydration status (Fig. 4). As with the urinary changes, the greatest increases in papillary urea and Na concentrations with LVP infusion occurred in the water-loaded rats, and the least in the hydropaenic group. Furthermore, the component changes in papillary water, urea and Na contents were similarly greatest in the water-loaded group (Fig. 5). Indeed, a significant decrease in papillary water content occurred only in the water-loaded group, in which the values after LVP infusion were not significantly different from those in both the normally hydrated and hydropaenic rats (Fig. 5). In hydropaenic rats, any increases in urea (non-significant) and Na (barely significant) contents were small.

The characteristic feature in rats with high solute excretion was that LVP infusion caused, at the most, minimal (and non-significant) changes in solute concentrations (Fig. 4) and in water and solute contents (Fig. 5). This applied not only in the mannitol experiments, where a similar lack of effect of LVP on urinary flow and composition (Figs. 2 and 3) was evident, but also in the saline experiments, where LVP did have a significant, though small, effect on the urine (Figs. 1 and 3).

Considering the data in all five groups of rats together, the following additional observations may be made.

(*a*) Papillary urea was far more labile than Na (Fig. 6).

(*b*) Papillary urea concentrations and contents were inversely related to urinary flows, but not related to the rates of solute excretion (cf. Figs. 4

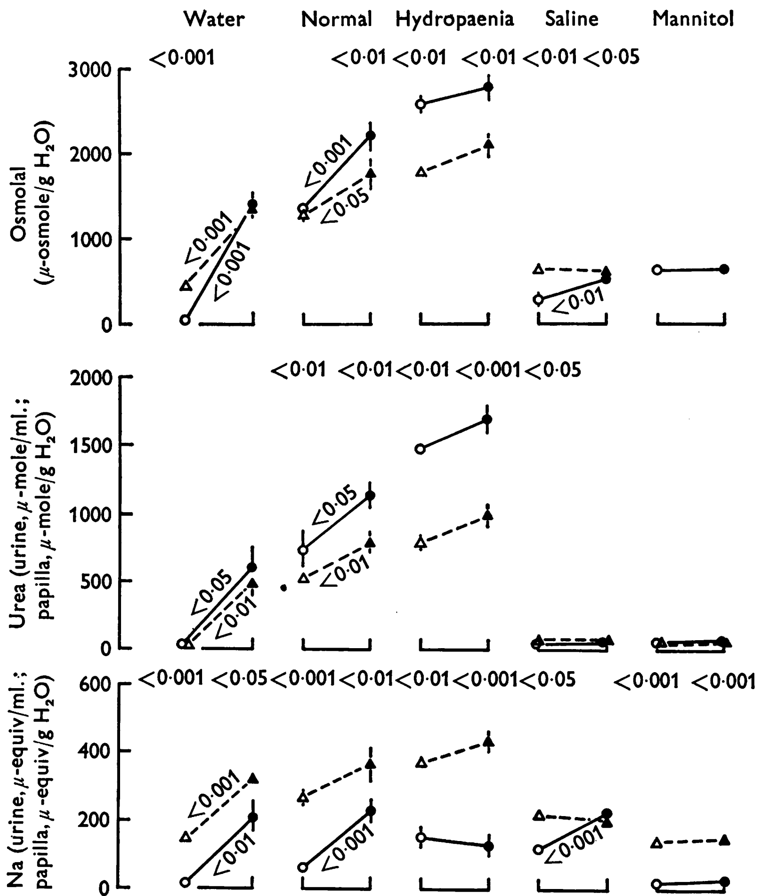

Fig. 4. Mean (± S.E.) urinary and papillary tip osmolal, urea and sodium concentrations in water-loaded, normally hydrated, hydropaenic, saline-loaded and mannitol-loaded rats. Values represent data from final periods in control experiments (urine ○; papillary tip △) and in experiments with lysine-vasopressin infusion, 60 μ-u./min. 100 g body wt. (urine ●; papillary tip ▲). *P* values along the lines represent the probability of the difference between control and LVP experiments being zero; and values at the top of each section represent the probability (paired *t* test) of the mean urine-papilla concentration difference being zero. Only values for $P < 0·05$ are included.

Note. The calculated values for papillary tip osmolality (see Methods) are regarded as approximations, only; and are presented merely in order to indicate the probable patterns of change. Calculated values were not obtained for the mannitol experiments, since tissue mannitol was not analysed.

and 5 with Fig. 3); they did, however, vary with urinary urea concentration (Fig. 6).

(*c*) Any differences in K and NH_4 concentrations were ascribable, mainly, to differences in water content, since only small, non-significant, differences in papillary content were observed (Fig. 5).

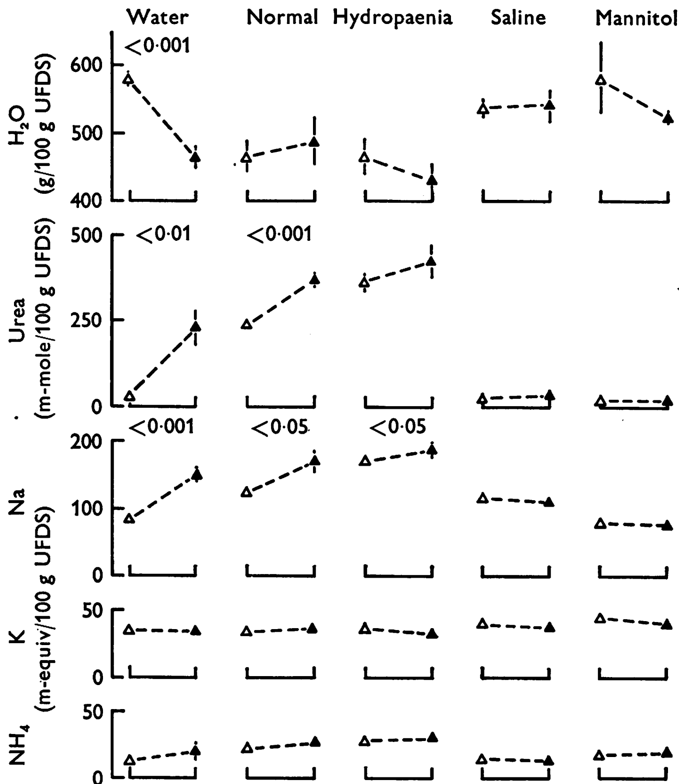

Fig. 5. Mean (± S.E.) papillary tip water, urea, sodium, potassium and ammonium contents in water-loaded, normally hydrated, hydropaenic, saline-loaded and mannitol-loaded rats. Values represent data from final periods in control experiments (△) and in experiments with lysine-vasopressin infusion, 60 μ-u./min.100 g body wt. (▲). *P* values represent the probability of the difference between control and LVP experiments being zero; only values for $P < 0.05$ are included.

Papillary-urinary solute concentration differences

The data in Fig. 4 enable a comparison to be made between urinary and papillary tip solute concentrations. This is informative in considering the renal transport mechanisms operative in the various circumstances.

In the diuretic control groups the very low urea concentrations in the

papilla were similar to those in the urine, although in the saline-infused rats the value in the papilla slightly, but significantly, exceeded that in the urine. In the control rats where urea concentrations were much higher (normal hydration and hydropaenia), urinary values were significantly higher than those in the papilla (Fig. 4).

Any influence of LVP infusion on the papillary-urinary urea concentration difference varied with the rate of solute excretion. In the saline- and mannitol-loaded rats, any difference between papillary and urinary urea concentrations remained small. However, in the three groups with low solute excretion rates, the relative increases in urinary and papillary concentrations with LVP infusion were such that the urinary-papillary difference was greatest in the hydropaenic group, and smallest (and non-significant) in the water-loaded rats (Fig. 4).

In all control groups, Na concentrations in the papilla were significantly higher than those in the urine. The changes in papillary and urinary Na concentrations induced by LVP infusion were such that, with one exception, the papillary-urinary difference in Na concentration remained large; the exception concerns the effect in saline-loaded animals where a significant increase in urinary concentration, coupled with the absence of any significant change in papillary concentration, resulted in the abolition of any significant papillary-urinary difference (Fig. 4).

DISCUSSION

The present results confirm that the urinary responses to administration of LVP vary with concurrent hydration and with the rate of solute excretion. The data show further that these differences in urinary responses are accompanied by differences in papillary responses. It may be concluded, therefore, that while some of the factors suggested by others (see Introduction) may be involved, the variable urinary responses to ADH are also dependent on differences in the medullary osmotic force responsible for passive water reabsorption from the collecting duct. This conclusion is valid irrespective of any reservations concerning the accuracy of the method used here to calculate tissue osmolality (see below), since the differences in the effect of LVP on individual solute concentrations may be interpreted in the same way.

The factors responsible for the effects of hydration (Atherton *et al.* 1968*b*; Hai & Thomas, 1969), mannitol loading (Atherton *et al.* 1968*a*) and saline loading (Atherton *et al.* 1970*a*) on medullary composition in the rat have been discussed in detail previously. Accordingly, the present discussion will be limited to considering the extent to which differences in papillary composition may be considered a complete explanation of the

varied urinary responses to LVP; and whether the data provide additional information concerning the interpretation of tissue slice analyses.

Tissue slice analysis

The urinary and tissue data available from the present and preceding (Atherton *et al.* 1971) papers cover a variety of experimental circumstances. The relations summarized in Fig. 6 are presented for three main purposes.

1. They show the wide range in tissue composition associated with that in urinary flow and composition. The variation in tissue urea concentration is far wider than that for either Na concentration or water content. The ability to elaborate highly concentrated urine is closely dependent on medullary accumulation of urea (Berliner, Levinsky, Davidson & Eden, 1958).

2. Over-all tissue composition is the resultant of the volume and composition of several component compartments. However, in the steady-state data presented in Fig. 6, urea concentrations in the papillary tip slice approach those in the urine; thus (as shown directly by micropuncture data) compartments outside the collecting duct must contribute. Furthermore, although changes in the flow and Na concentration of fluid in the collecting duct must contribute to changes in slice composition, wide differences in papillary Na concentration may be observed at any urinary Na concentration, and differences in papillary water content at a particular urine flow (Fig. 6).

These observations support previous conclusions (see Atherton *et al.* 1968*a*, Atherton *et al.* 1970*a*) that papillary slice composition is determined, mainly, by compartments outside the collecting duct.

3. At high urinary osmolalities, calculated papillary osmolalities are consistently lower than expected. Several explanations are possible: (*a*) the calculation does not include the contribution of other osmotically active medullary solutes, such as amino-acids (Robinson, Owen & Schmidt-Nielsen, 1966); (*b*) the osmotic force driving water reabsorption from the collecting duct may be that of a localized sub-compartment of medullary interstitial fluid (Marsh, 1970); (*c*) the larger the papillary slice, the larger will be the deviation from true papillary tip composition, particularly where highly concentrated urine is elaborated. Because of these uncertainties, the calculated values in Fig. 4 are presented merely in order to show the probable patterns of change in papillary tip osmolality.

In any event, it is concluded that where incomplete osmotic equilibration appears to occur in the present experiments, this probably underestimates the real situation.

Effects of hydration at low solute excretion rates

The well known rapidity of the effect of ADH on urine flow and osmolality is due to the rapid increase in nephron permeability to water (White & Rolf, 1965; Atherton *et al.* 1971). If the collecting duct permeability to water were rendered sufficiently high to permit complete osmotic equilibration, the urinary osmolality achieved would depend, completely, on the prevailing medullary osmolality. Although it is presumed that endogenous release of ADH was near minimal in the water-

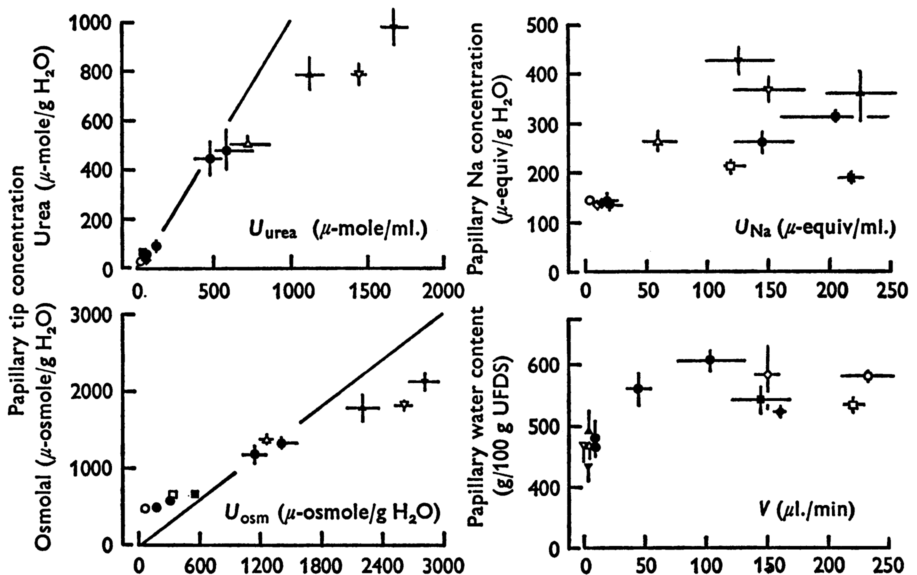

Fig. 6. Relations between *top left*, papillary tip and urinary urea concentrations; *top right*, papillary tip and urinary sodium concentrations; *bottom left*, calculated papillary tip osmolality and urinary osmolality; and *bottom right*, papillary tip water content and urinary flow. Values, presented as means (± S.E.), all represent essentially steady-state conditions: normally hydrated, controls (△) and LVP (▲); hydropaenic, controls (▽) and LVP (▼); saline-loaded, controls (□) and LVP (■); mannitol-loaded, controls (◇) and LVP (◆); and water-loaded, controls (○) and LVP (●). Data for lysine-vasopressin infusion rates at 2·5, 5, 15 and 60 μ-u./min. 100 g body wt. in water-loaded rats are derived from the preceding paper (Atherton *et al.* 1971); data for the other experiments are from the present paper. The continuous lines represent equal concentrations in papilla and urine.

Note. In the top left section, values for experiments on saline- and mannitol-loaded rats are not plotted, since they would be obscured by the low values in corresponding LVP experiments.

Tissue osmolal concentrations were not calculated in mannitol experiments (see note to Fig. 4).

loaded group and near maximal in the hydropaenic rats, there seems no reason to suppose that the extent of osmotic equilibration differed after LVP infusion, since the dose (60 μ-u./min. 100 g body wt.) produced near-maximal effects in the water-loaded animals (Atherton *et al.* 1971). The differences in the urinary responses to prolonged infusion may be attributed, therefore, to differences in the osmotic force capable of being generated under the particular experimental conditions.

The present data show that the greater the urinary osmolality at the start of LVP infusion, the smaller were any increases in papillary solute concentrations even on prolonged administration. Whatever factors may be responsible for the differences in medullary composition in the variously hydrated control groups (see Hai & Thomas, 1969), it seems reasonable to conclude that the antidiuretic response to LVP will vary with the collective effect of other factors on the transport characteristics of the nephron; and on the mechanisms influencing the ability to generate a steep cortico-medullary osmolal gradient.

Effects of high solute excretion rate

As discussed above, the increased urinary osmolality resulting from LVP infusion in water-loaded animals, with low rates of solute excretion, is attributable both to increased nephron permeability to water and to an increased medullary reabsorptive force. Yet, although prolonged infusion of LVP caused the restitution of relatively steep solute concentration gradients in these water-loaded rats, this did not occur during saline and mannitol loading. It must be concluded that the experimental circumstances and intrarenal changes associated with the high rates of solute excretion were such that a high dose of LVP was incapable of reducing papillary water content and increasing papillary urea and Na contents even over prolonged periods.

The mechanisms responsible for this restricted responsiveness of medullary composition to LVP are uncertain, but there seems no reason to suppose that this is due to inhibition of the effects on the various LVP-sensitive loci discussed in the previous paper (Atherton *et al.* 1971). It seems more economical to conclude that any influence of LVP on factors determining the medullary contents of water and solute are obscured by the large changes in medullary blood flow and intraluminal fluid flow induced by mannitol (Atherton *et al.* 1968*b*; Seely & Dirks, 1969) and saline (Earley & Friedler, 1965; Landwehr, Klose & Giebisch, 1967; Atherton *et al.* 1970*a*) loading.

The limited increase in urinary osmolality during LVP infusion in the saline-loaded group, and the absence of any significant change in the mannitol-infused rats, may also depend on a further factor: incomplete

osmotic equilibration across the collecting duct. For reasons discussed above, this is regarded as a real phenomenon in the saline-loaded group. There seems no reason to postulate that this is due to an inhibition of the effect of LVP on nephron permeability to water; or to suppose that the high dose of LVP was inadequate to ensure maximal collecting duct permeability to water. Indeed, the occurrence of small, but significant, decreases in urinary flow and in free-water clearance shows that an increase in water permeability did occur in the saline-loaded group; while the absence of such significant changes in the mannitol-loaded rats would be expected if hypertonic mannitol caused a high rate of release of endogenous ADH, with high nephron permeability to water, even in the control group. For these reasons, we agree with the suggestion (Berliner & Bennett, 1967) that where water delivery from the distal tubule is increased sufficiently during high rates of solute excretion, then osmotic equilibration may remain incomplete despite maximal collecting duct permeability to water, particularly if the medullary osmotic reabsorptive force is low.

In summary, we conclude that the differences in the antidiuretic response to LVP associated with varying hydration and varying solute excretion depend, to a large degree, on differences in renal medullary composition. In view of this, we speculate that differences in (*a*) the maximal capacity to elaborate concentrated urine in a variety of circumstances, and (*b*) the dose–response relation for ADH with varying solute excretion (Orloff, Wagner & Davidson, 1958) depends on differences in the papillary osmolality under the prevailing conditions.

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