The Action of Pitressin on Solute Permeability of the Rabbit Nephron in Vivo

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ABSTRACT The isotopic equilibration of urea, thiourea, and inulin between urine and plasma was determined in rabbits in the presence or absence of antidiuretic hormone (ADH). Animals were anesthetized with ethanol and permitted to reach steady state after completion of surgery. Tracer was then administered by intraarterial infusion in such a manner that a high constant specific activity in plasma was rapidly attained. Urine flow was kept independent of ADH by addition of mannitol. Urea/creatinine clearance ratios and the accumulation of urea in renal medulla and papilla also remained unaffected by ADH. Under these conditions, thiourea and inulin at all times approached equilibrium at similar rates. In the absence of ADH, urea also equilibrated at a rate similar to that of inulin. The addition of ADH, however, significantly prolonged the delay before urinary urea reached the high constant specific activity of plasma urea. These observations are interpreted in terms of a specific effect of the hormone on the solute permeability of the nephron.

INTRODUCTION

The response of epithelial membranes to the action of the antidiuretic hormone of the pituitary gland (ADH) has been investigated in considerable detail by an analysis of such isolated systems as the toad skin and bladder (1, 2). In particular, the work of Leaf and Hays (3) showed that ADH increases the permeability of the toad bladder not only to H_2O, but that it also specifically alters the transmembrane flux of various solutes. Thus, the flow of urea across the toad bladder epithelium is significantly accelerated by ADH, whereas no such effect is seen with thiourea.

The evidence for similar actions of the hormone in vivo remains more indirect. Jaenike (4) compared the urea content of the renal medulla in dogs undergoing water diuresis or treated with mannitol plus ADH and concluded that the hormone exerts an effect on the permeability of the collecting duct to urea. Thomas (5) placed a similar interpretation on the results of his analysis

of the washout and reaccumulation of urea during diuresis and antidiuresis. Bray (6) in the hydropenic dog observed a ratio of urea concentrations in urine and papilla water of 1.5, compared with a similar ratio for thiourea of 9. This finding suggests that in the presence of ADH, urea but not thiourea can diffuse across the wall of the collecting duct. In the rat, ADH has been reported to affect H_2O but not net urea flux out of the distal convoluted tubule (7). The present experiments aimed at a clarification of the effects of ADH on the solute permeability of the mammalian nephron under physiological conditions.

METHODS

A technique was recently described which permits the study of the isotopic equilibration of plasma and urine under steady-state conditions (8). Essentially the method consists of the passage through the kidney of what might be described as a square pulse of arterial tracer concentration and the simultaneous determination of the rate at which the specific activity of a solute in urine approaches the constant specific activity of this solute in arterial plasma. Partial reabsorption of a filtered solute will not alter its specific activity in the urine. The appearance of high specific activity filtered solute in the urine may be delayed, however, by exchange of urinary labeled solute with the contents of an unlabeled tissue pool. Among the chief factors determining the rate and extent of such equilibration are the size of the tissue pool and the permeability of the nephron.

In the present paper this principle provides the basis for the study of the effect of ADH on the permeability of the nephron to different solutes. The preparation of the animals and the infusion procedures have been previously described in detail (8). At least 60 min were allowed to elapse after completion of surgery in order to permit the animals to reach steady state in terms of urine flow and composition. At that time the tracer contained in the mixing chamber was added to the arterial infusion. The composition of the infusion fluids is given in the legend to Fig. 2. The secretion of endogenous ADH was suppressed and anesthesia was induced by slow intravenous administration of 3 to 5 ml ethanol. Anesthesia was maintained by inclusion of ethanol to a level of 5% in the sustaining infusion. The intravenous infusions were kept hypotonic to plasma. It was not possible to produce in our rabbits a pure water diuresis adequate for the rapid urine sampling required. Even in the control animals when the excretion of solute was to be measured in the absence of ADH, 2.5% mannitol had to be added to the intraarterial infusion. The total osmotic load administered to these animals, however, remained less than the isosmotic equivalent of the volume of fluid infused. When the effect of ADH was measured, the hormone (Pitressin, Parke, Davis and Co.) was added to the arterial infusion to a concentration equivalent to a dose of 1 milliunit/kg body weight/min. At the same time the concentration of mannitol was raised to 5% in order to keep the urine flow constant. The glomerular filtration rate as previously was equated with creatinine clearance. Chemical assay of urea was performed with the diacetyl-monoxime method (9), and that of inulin by the diphenylamine procedure of Rolf, Surthshin, and White (10). The radioassay of urea and thiourea (products of Volk Radiochemical Co., and Calbiochem Corp., respectively)

involved counting of samples at constant thickness on a Nuclear-Chicago gas flow counter before and after incubation with purified urease (Worthington) for a period sufficient to decompose over 99% of the urea originally present. The samples were then acidified before drying on the planchets. Extracts for radioanalysis of tissues after blotting and weighing were prepared by suspension of the sliced sample in 0.5% carrier solution in distilled water, freezing, and thawing, followed by continued extraction overnight in the cold. The suspension was then quickly heated to 100°C, cooled, and cleared on the centrifuge. For chemical assay, carrier was omitted.

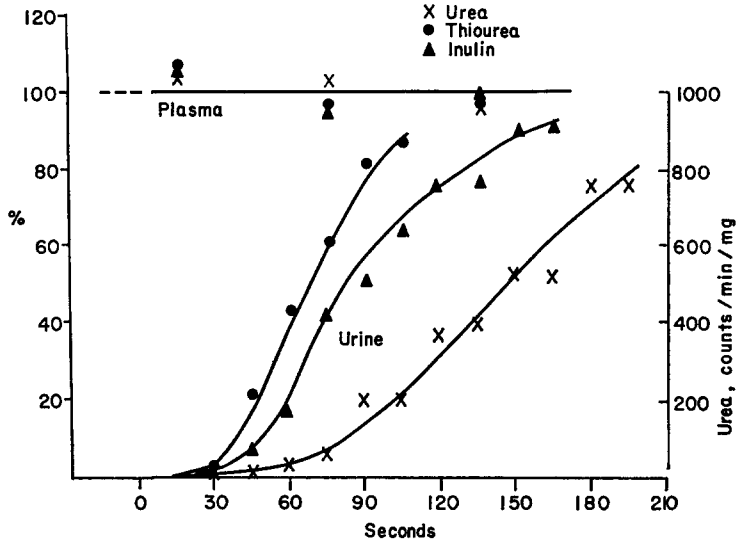

FIGURE 1. Equilibration of inulin, thiourea, and urea in the presence of ADH. Animal TU No. 31: 3.2 kg. Results shown for right kidney; urine flow 0.75 ml/min, urea clearance 6.1 ml/min, creatinine clearance 8.4 ml/min. For infusions etc., see Fig. 2, top. Abscissa, time from initial urine collection, starting 90 sec after beginning of tracer infusion. Ordinate, concentration of inulin, thiourea-^{14}C: for plasma, as % of mean; for urine, as % of final equilibrium concentration determined between 180 and 240 sec. For reasons discussed in text, urea values are expressed as counts/min/mg.

RESULTS

In Fig. 1 are shown the results of a representative experiment in which inulin, thiourea-^{14}C, and urea-^{14}C were infused into an ADH-treated animal at steady state. It will be seen that both inulin and thiourea-^{14}C approached a final equilibrium concentration in urine within less than 2 min after their first appearance. During such short periods little breakdown of thiourea is expected. Moreover, since the animals are in steady state, concentration of ^{14}C and specific activity will vary directly with one another. The use of radioisotope concentration rather than specific activity appears justified, therefore; it further circumvents both the difficulty of thiourea assay and the uncertainty deriving from the fact that equilibration of this compound between

plasma and red cells is far from instantaneous (11). The specific activity of thiourea in the plasma perfusing the kidneys may therefore significantly exceed that in the plasma separated from blood obtained through the collecting

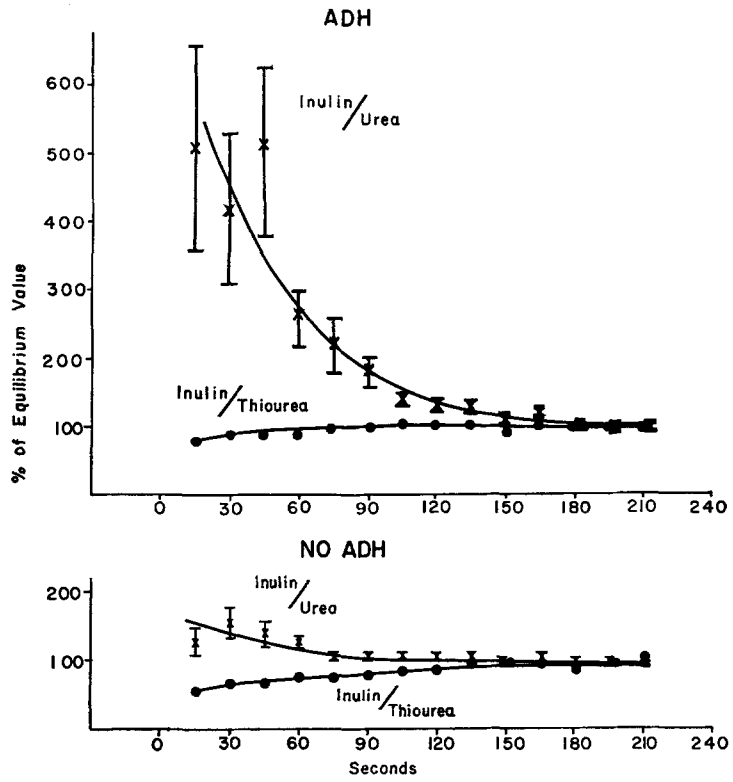

FIGURE 2. Effect of ADH. Abscissa, as for Fig. 1. Ordinate, concentration ratios of inulin/urea-^{14}C and inulin/thiourea-^{14}C in urine, as % of final equilibrium value. Animals weighing approximately 3 kg were anesthetized by slow intravenous administration of 3 to 5 ml ethanol. Through a marginal ear vein a continuous infusion was administered of 0.115 M NaCl + 12 mM creatinine in 5% ethanol at a rate of 1.2 ml/min. The intra-arterial infusion (1.2 ml/min) contained 2.5 mg/ml each of urea and thiourea; for experiments in this figure (top) 50 mg mannitol + 2.5 milliunits ADH were further added/milliliter of infusion. In the absence of ADH (bottom) the mannitol concentration was reduced to 25 mg/ml. The mixing chamber contained 5 μc each of urea and thiourea, plus 70 mg inulin in 3.5 ml of the appropriate intraarterial infusion. Values shown are the mean ± SEM calculated from eleven experiments with ADH, and fourteen experiments without ADH. SEM for inulin/thiourea ratios did not exceed ±10%.

catheter. The equilibration rate of thiourea as calculated here is independent, however, of plasma specific activity, provided the latter does remain constant throughout the experiment. That an adequate constancy of plasma concentrations was achieved can be seen in Fig. 1. Unlike thiourea-^{14}C and inulin, urea-^{14}C required longer than 2 min under the conditions of Fig. 1 to reach

constant concentration or specific activity in urine. The ease of urea assay here permits the ready determination of specific activities.

A total of eleven similar studies on the equilibration of urea, thiourea, and inulin in the presence of ADH was carried out. The relative equilibration rates are collectively expressed in Fig. 2 (top) as the inulin/thiourea-^{14}C or inulin/urea-^{14}C concentration ratios in urine, normalized as per cent of the final equilibrium value. As already seen in Fig. 1, thiourea reaches equilibrium a little faster than inulin, so that the ratio of inulin to thiourea initially falls somewhat below 100%. Urea on the other hand clearly equilibrates more slowly than inulin (Fig. 1). Accordingly, in Fig. 2 (top) the initial inulin/urea values greatly exceed 100%.

In the ADH experiments collected in Fig. 2 (top) the mean plasma urea level was 6.7 μmoles/ml, the urea/creatinine clearance ratio 0.74 (range 0.61 to 0.93), and the urine flow 0.8 ml/min (range 0.6 to 1.0). To study solute equilibration in the absence of ADH but at similar values for urine flow and urea/creatinine clearance ratios it was necessary to reduce the mannitol concentration of the arterial infusion to 2.5%. The results of fourteen such studies are collected in Fig. 2 (bottom). Here the mean plasma urea level was 6.3 μmoles/ml, the urea/creatinine clearance ratio 0.69 (range 0.41 to 0.91), and the urine flow 0.7 ml/min (range 0.5 to 0.8). It is apparent that in contrast to the situation when ADH is present, equilibration of urea now proceeds almost as fast as that of inulin. The excretion of thiourea-^{14}C in relation to that of inulin remains essentially unchanged.

The large differences in relative equilibration rates of urea seen in Fig. 2 (top and bottom) were obtained under conditions in which urine flow and urea/creatinine clearance ratios remained reasonably constant. To determine whether, in spite of this constancy, the administration of ADH might have altered the amount of solute trapped in the renal medulla and papilla, tissue obtained by removal of cortex from six kidneys of animals represented in Fig. 2 (top) and from ten kidneys of animals not treated with ADH (Fig. 2, bottom) was analyzed for urea at the end of the experiments. The values found were 25 μmoles/g wet weight tissue (range 20 to 40) in the absence of ADH, and 35 μmoles/g (range 28 to 45) after administration of the hormone. In another series of experiments in which urea was infused intravenously, six kidneys were obtained from control animals (no ADH) and the same number from experimental animals (after ADH administration). Each tissue was analyzed in triplicate. The following mean urea concentrations were found, in micromoles per gram wet weight medulla plus papilla: control, 23 (range 15 to 32), experimental, 22 (range 13 to 27). Under present conditions, therefore, ADH infusion has little effect on urea accumulation in the kidney. Similarly, no significant influence of ADH could be detected on the retention of radioactivity in the kidney of rabbits infused with thiourea-^{14}C over a period of 90 min. However, chromatographic analysis of such tissue extracts

demonstrated that not all the radioactivity was contained in the thiourea spot. The possibility of some effect of ADH on the size of the thiourea tissue pool under our conditions can therefore not be entirely excluded.

DISCUSSION

The present report compares the relative rates of equilibration of urea and thiourea in plasma and urine in relation to the equilibration of inulin. Inspection of Fig. 2 shows that independently of the presence of ADH, thiourea and inulin reach equilibrium at similar rates. Actually, in most cases thiourea slightly preceded inulin. The full significance of this finding is not clear but it does indicate that some thiourea apparently enters the urine through the tubules. In sharp contrast with thiourea, the equilibration of urea between plasma and urine is strongly influenced by the action of ADH. Even in the absence of exogenous hormone, some delay in the equilibration of urea compared to that of inulin is apparent. This delay becomes much more pronounced after administration of ADH. The effect of ADH is seen in spite of the fact that urine flow, the fraction of filtered urea excreted, and the size of the urea pool in the renal medulla all remained reasonably constant.

The high medullary urea concentration presumably results from the action of a countercurrent mechanism, similar to that described e.g. by Aukland and Berliner (12). A further consequence of the functioning of a countercurrent system will be a slowing down of the direct labeling of the trapped urea through the blood circulation. Evidence for the absence of significant direct labeling is provided by the fact that neither in our experiments nor in the earlier studies of Chinard and Enns (13) was there any precession of labeled urea in urine over suitable glomerular markers. It follows then that the pool, initially unlabeled under present conditions, acquires its label mainly from urinary urea. The delayed equilibration of urea between urine and plasma in the presence of ADH, therefore, presumably reflects the exchange of labeled urea for unlabeled pool urea across the tubular epithelium. In other words, ADH appears to increase the permeability of the nephron to urea. The likelihood of significant net flux of unlabeled urea occurring from the pool into tubular fluid, as described by Lassiter, Mylle, and Gottschalk (14) in nondiuretic animals, seems small under present conditions of strong diuresis.

The argument here adduced in support of an ADH effect on permeability of the nephron to urea ignores the likelihood that control and ADH-treated animals will differ in the relative proportions of proximal and distal bulk reabsorption. Indeed, the high mannitol concentration in animals infused with ADH will presumably cause a greater volume of tubular fluid and therefore also a larger amount of urea to be presented to the distal tubule than in the case of control animals. This fact in turn implies that more net reabsorp-

tion of urea will occur in the distal nephron of ADH-treated animals than in that of controls. However, reabsorption does not alter the specific activity of urea remaining in the tubule, so that changes in the locus of reabsorption are not expected to affect the isotopic equilibration rate of urea in plasma and urine. In any case, the infusion of ADH and mannitol delays this equilibration in spite of the fact that such a treatment may increase the amount of labeled, filtered urea reaching the distal portions of the nephron.

Another assumption implicit in the interpretation of our data is that of the absence of changes in medullary blood flow upon administration of ADH under present conditions. Some justification for this assumption is found in the invariance of the urea concentration in the medulla of control and of ADH-treated animals.

The lack of any effect of ADH on thiourea equilibration leads to the inference that the permeability of the nephron to thiourea is not altered by the hormone. The same conclusion was drawn by Leaf and Hays (3) from their work on the toad bladder. The good agreement between in vitro and in vivo studies lends credence to the interpretation of results obtained by the experimental approach used here for the analysis of ADH action under physiological conditions.

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