Fate of vasopressin perfused into nephrons of Wistar and Brattleboro (diabetes insipidus) rats

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Summary

1. Iodinated vasopressin was microinjected into early proximal or distal tubules of Wistar and Brattleboro (diabetes insipidus) rats. Sites of infusion were determined by the lissamine green transit time method.

2. Urinary recovery of \(^{125}\)I after proximal and distal injections was 89 ± 1.7% and 94 ± 1.0% in Wistar rats (corrected for inulin) and 81 ± 2.0 and 92 ± 2.0% in Brattleboro rats (uncorrected); injection of hormone into vascular stars resulted in similar \(^{125}\)I recoveries from punctured and contralateral kidneys.

3. Radioactive substances excreted after perfusing proximal and distal sites in Brattleboro animals, and \(^{125}\)I-labelled hormone added to urine from the contralateral kidney, bound similarly to a specific arginine vasopressin antiserum and demonstrated similar radioactive elution profiles after passage through Sephadex G25 columns.

4. Incubation of labelled and unlabelled vasopressin with rat kidney homogenates resulted in similar and complete degradation of the hormone.

5. Results indicate that most of the vasopressin injected into either proximal or distal nephrons enters the urine intact, and no evidence of tubular secretion was found when perfusing vascular stars. Enzymes in rat renal tissue degrade labelled vasopressin, but the ability of the proximal tubule to hydrolyse the \(^{125}\)I-labelled vasopressin is limited, especially when compared with that reported for several linear peptide hormones.

Key words: antidiuretic hormone clearance, arginine vasopressin, kidney, radioimmunoassay, renal micropuncture.

Introduction

The manner in which vasopressin is excreted into the urine is unclear. Whereas many (Towbin & Ferrell, 1963; Harvey, Jones & Lee, 1967; Lauson, 1974; Shade & Share, 1977; Rabkin, Share, Payne, Young & Crofton, 1979) believe that the source is glomerular filtration, others (Walter & Bowman, 1973) suggest that only distally secreted vasopressin enters the urine intact. favouring the latter view are observations (Pullman, Oparil & Carone, 1975; Carone, Pullman, Oparil & Nakamura, 1976; Peterson, Oparil, Froulet & Carone, 1977) that angiotensin and bradykinin, peptides with molecular weights similar to that of vasopressin, are extensively degraded when infused into proximal tubules of rats, to an extent that if vasopressin were metabolized similarly virtually all of the vasopressin appearing in the urine would have to be secreted at a distal site in the nephron.

The present study, performed on Wistar and Brattleboro (diabetes insipidus) rats, was designed to help clarify mechanisms of renal tubular handling of filtered vasopressin. \(^{125}\)I-labelled vasopressin was microperfused into renal proximal and distal tubules as well as into vascular stars, and labelled peptides in the urine were characterized by radioimmunoassay and gel chromatography.
Methods

Wistar (200–250 g) or Brattleboro (150–200 g) rats were anaesthetized with pentobarbital and prepared for micropuncture as previously described (Grandchamp, Scherrer, Scholer & Bornand, 1975). Briefly, an infusion of mannitol (278 mmol/l) and sodium chloride (154 mmol/l) was administered at a rate of 2 ml h\(^{-1}\) 100 g\(^{-1}\) body weight throughout. The ureters were cannulated and the left kidney was stabilized in a Lucite chamber filled with mineral oil.

Micropuncture sites were identified by the transit time technique of Steinhausen (1963). The perfusate, containing lissamine green, was injected manually through pipettes with 5 \(\mu\)m tips, over a period of 2–7 min, care being taken to infuse the coloured test solution downstream only. In experiments designed to measure recovery of radioactive label in the urine, 40 nl of a physiological solution, containing iodinated arginine vasopressin, was microperfused at a constant rate proximally, distally or into vascular stars. Urine from the infused and contralateral kidneys was collected both during the infusion and for 10 min after its completion. In protocols designed to characterize the labelled urinary products, 120–240 nl was microperfused in a manner similar to that described above.

Each 40 nl of perfusate contained 4–7 attomol of \(^{125}\)I-labelled vasopressin, and in studies on Wistar animals the solution also contained 50 mCi of \(^{3}\)H-labelled inulin (New England Nuclear, Boston, Mass., U.S.A.). Concentrations of other solutes were: acetic acid, 10 mmol/l; sodium chloride, 120 mmol/l; sodium bicarbonate, 35 mmol/l; lissamine green, 5 mmol/l.

Arginine vasopressin (Ferring AB, Malmo, Sweden) was labelled with \(^{125}\)I (Eidgenössisches Institut für Reaktorforschung, Würenlingen, Switzerland) and purified as described previously (Czernichow, Merkelbach & Vallotton, 1975). Purity of the iodinated preparations (determined by chromatography followed by radioautography) was between 85 and 90% and their specific radioactivities (Morris, 1976) ranged from 634 to 1000 Ci/mmol. In preliminary experiments with thin-layer chromatography in several systems, combined with radioautography, we found that the \(^{125}\)I-labelled vasopressin and lissamine green in the preparation migrated separately.

Urinary recovery of microperfused \(^{125}\)I

Radioactivity was measured by counting the urine for 30 min in a \(\gamma\)-spectrometer (LBK-Wallac 1280, Ultraspectro, Turku, Finland). Samples were acceptable if the radioactive content of urine collected before injection and from the contralateral kidney during perfusion was similar to the background value. Recovery was determined by comparing radioactivity counts in the urine to mean radioactivity of a minimum of five perfusate samples prepared randomly throughout each study. \([\text{H}]\text{Inulin}\) was determined after \(^{125}\)I radioactivity was no longer detectable. The evaporated samples were dissolved in water, and portions were counted in a liquid scintillation spectrometer (Packard Instruments, Chicago, Ill., U.S.A.). There was no quenching, and the theoretical to actual \(\text{H}\) radioactivity ratio of standards prepared for an individual experiment was 0.98.

Characterization of the labelled urinary products

Radioimmunoassay. Portions (50 and 100 \(\mu\)l) of urine collected during perfusion of proximal and distal tubules were incubated for 24 h with a highly specific antiserum against vasopressin (Czernichow, Reinharz & Vallotton, 1974). In addition, perfusate was added to the contralateral urine of each sample in amounts calculated to simulate the radioactivity recovered from the microperfused ipsilateral kidney. (Antibody binding in perfusate added directly to urine was similar to that of blanks made with \(^{125}\)I-labelled vasopressin in the presence or absence of urine, as well as with and without lissamine green.) In our radioimmunoassay procedure (Czernichow et al., 1975) antibody-bound and -free \(^{125}\)I are separated with dextran-coated charcoal.

Gel filtration. Urine obtained after micro-perfusion of proximal and distal tubules, sodium chloride (154 mmol/l) standards and vasopressin added directly to rat urine were applied to Sephadex G25, 1.2 cm x 25 cm columns, and eluted with acetic acid (0.02 mol/l). One hundred and fifty 2 ml fractions were collected at a flow rate of 2 ml/h at 4°C.

Enzymatic inactivation of \(^{125}\)I-labelled vasopressin by rat kidney homogenate

Studies were also performed to determine if rat renal enzymes were capable of metabolizing the iodinated vasopressin used in these experiments. Kidneys removed from Wistar rats were homogenized (10%, w/v) in phosphate buffer (0.1 mol/l, pH 7.2) and centrifuged at 50 000 g for 40 min at
Vasopressin handling by renal tubule

4°C. Then 200 μl of supernatant was incubated at 37°C with 10–20 μl of 125I-labelled vasopressin for either 3 or 60 min. Reactions were stopped by placing the tubes in boiling water for 10 min. Recovery of 125I-labelled vasopressin was estimated by chromatography combined with radioautography, or by radioimmunoassay. Labelled hormone added to buffer, with or without boiled supernatant, served as control.

Results

Recovery of 125I label in the urine

Recovery of [3H]inulin was 91.4 ± SEM 1.4% in proximal tubules, compared with 98.1 ± 1.3% distally, suggesting that despite precautions some retrograde flow occurred in the proximal tubules. Corrected recoveries of 125I were 89 ± 1.7 and 94 ± 1.0% in 11 proximal and 14 distal tubules respectively.

Recovery of 125I from 13 proximal and 11 distal tubules from Brattleboro rats with hypothalamic diabetes insipidus was 81 ± 2.0 and 92 ± 2.0% respectively (Fig. 1). These results, though uncorrected for inulin, are similar to those in Wistar rats, and validate the use of Brattleboro animals in experiments designed to characterize the labelled urinary peptides.

Six vascular stars in four Wistar rats were also perfused. There was no significant difference in radioactivity recovered in the urine from injected (21 ± 0-9%) and contralateral (20 ± 0-7%) kidneys, the average ratio being 1-05 (range 0.99–1.10).

Characterization of the labelled urinary product(s)

Radioimmunoassay. The amount of endogenously produced vasopressin excreted by Wistar rats, even when hydrated, is much greater than the small quantities of labelled vasopressin perfused in these experiments. Under such conditions endogenous (unlabelled) hormone competes with the 125I-labelled vasopressin for binding, making precise measurement of the small amount (10–40 attomol) infused difficult by current radioimmunoassay techniques. For this reason, immunological identification of urinary labelled products was not attempted in Wistar animals. However, homozygous Brattleboro rats with hereditary diabetes insipidus produce no endogenous vasopressin (Valtin, Sokol & Sunde, 1975) and any vasopressin in their urine is that injected.

Enzymatic inactivation of 125I-labelled vasopressin by rat kidney homogenate

The surprising observation that virtually all the hormone perfused was recovered, seemingly contrasted with observations that renal tissue metabolizes both unlabelled and [3H]glycinamide vasopressin (Walter & Bowman, 1973; Lauson, 1974). Advantage was taken of this fact to assess the labelled urinary product(s) as if they were tracers in a standard radioimmunoassay.

Antibody-binding studies were performed on urine collected after perfusing 10 proximal and 11 distal tubules in three Brattleboro rats. Binding averaged 49.5 ± 2.0% after proximal tubular perfusion, compared with 48.9 ± 1.3% in the controls (perfusion added to urine from the contralateral kidney). Similarly, urine obtained after distal punctures and their controls were 48.8 ± 1.9 and 48.4 ± 1.1% respectively.

Gel filtration. Urine collected after microperfusion of proximal and distal tubules was filtered on a Sephadex G 25 column. The elution profiles, which demonstrated two peaks (Fig. 2), were similar to the control (125I-labelled vasopressin added directly to urine). The major peak contained 72, 76 and 83% of the total radioactivity in proximal, distal and control samples respectively. The nature of the small second peak which eluted after the void volume is unclear, as it was not present when 125I-labelled vasopressin containing sodium chloride (154 mmol/l) standards (not shown) were filtered through the column. This small peak, however, could be due to complexing of vasopressin to macromolecules, a phenomenon known to occur in urine (Lauson, 1974).
Fig. 2. Gel chromatography, on a Sephadex G 25 (1.2 cm x 25 cm) column, of urine collected after perfusing proximal and distal tubules of Brattleboro rats. Elution with acetic acid (0.02 mol/l) produced similar radioactive profiles whether the hormone was perfused proximally (○), distally (O) or added directly to urine excreted by the contralateral kidney (△).

Discussion

Biologically and immunologically assayable antidiuretic hormone is found in the urine (Lauson, 1974; Musolan & Campbell, 1975; Walter & Schlank, 1975; Rabkin et al., 1979) and prompted experiments designed to determine if enzymes in rat kidney homogenate would inactivate the iodinated vasopressin utilized in our protocols. In preliminary studies, with radioimmunoassay, the homogenate inactivated 70 and 100% of unlabelled vasopressin after incubation for 3 and 60 min respectively. Similarly, antibody binding of iodinated vasopressin decreased 78% at 3 min and 100% at 1 h. Radioactivity from iodinated hormone incubated with native supernatant migrated with $R_f$ 0-42, and the $R_p$ was 0-13 for $^{125}$I-labelled vasopressin incubated with or without boiled supernatant.

Recovery of label was 89% after proximal injection and 94% after distal perfusion. This does not demonstrate reabsorption of vasopressin, however, since purity of our preparation was only 85–90%. Results from radioimmunoassay and gel-filtration experiments clarified the nature of these labelled product(s) by demonstrating that virtually all the urinary radioactivity resided in a compound(s) whose immunological properties and/or molecular weight were similar to that of arginine vasopressin. We also analysed some samples by thin-layer chromatography followed by radioautography. Radioactive material in urine collected after proximal or distal tubular punctures, and $^{125}$I-labelled vasopressin added to urine from the contralateral kidney, migrated similarly. Taken together, these data demonstrate that the fate of vasopressin perfused into the renal tubule is quite different from that of the linear peptide hormones angiotensin and bradykinin, in that vasopressin is quite resistant to the hydrolytic action of proximal tubular brush-border enzymes.

Data resembling ours have been reported for another neurohypophyseal hormone, oxytocin. Peterson et al. (1977) perfused proximal straight tubules from rabbit kidneys in vitro with [14C]-
angiotensin and [3H]oxytocin. The linear peptide was extensively degraded and reabsorbed and the neurohypophysial hormone remained intact. These authors cited evidence suggesting that the resistance of oxytocin to enzymatic cleavage is due to the cyclic part of the molecule (Hill & Smith, 1957; Walter & Hoffman, 1974). Vasopressin has a structure almost identical with that of oxytocin, differing from it by a single amino acid in the linear portion and one in the cyclic part of the molecule.

The kidney plays a primary role in the disposal of vasopressin. Data from experiments with tissue homogenates (Lauson, 1974; Musolan & Campbell, 1975; Walter & Schlank, 1975) and the isolated perfused kidney (Walter & Bowman, 1973; Rabkin et al., 1979) have demonstrated that vasopressin is extensively hydrolysed in the kidney. Renal inactivation is primarily by cleavage in the non-cyclic portion of the molecule (our antibody is most specific for the terminal amino acid sequence, Pro-Arg-Gly, the region where renal degradation takes place) (Walter & Bowman, 1973; Bauman & Dingman, 1976; Thomas & Lee, 1976). Such data, taken together with ours, suggest that filtered vasopressin escapes hydrolysis and is excreted intact in urine, but the hormone in postglomerular blood is extensively inactivated at other intrarenal sites. Consistent with this hypothesis are studies by Bauman & Dingman (1976), who injected 125I-labelled vasopressin into human volunteer subjects. The only labelled product entering the urine during the first 20–30 min after injection was intact hormone, but afterwards iodinated fragments could also be detected.

Several reservations deserve comment. The quantity of hormone perfused, although small, is still supraphysiological. Thus a reabsorptive mechanism with limited capacity might have remained undetected because its tubular maximum was but a small fraction of the injectate. Also, effects of lissamine green on brush-border hydrolytic enzymes have not been determined. Similarly, failure to uncover secretory activity by perfusing vascular stars does not rule out secretion entirely (although a large amount of secretory activity should have been evident) and further experiments (e.g. precession studies) are required to resolve this question. Finally, our data describe the fate of labelled vasopressin in the nephron and it is possible that unlabelled hormone might be handled differently (Shade & Share, 1976; Weitzman & Fisher, 1978).

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