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Influence of Antidiuretic Hormone on Intrarenal Blood Flow Distribution in Diabetes Insipidus Dogs and Rats[1] (39255)

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The influence of antidiuretic hormone (ADH) on intrarenal blood flow distribution has not been widely investigated. The studies of Fisher, Grunfeld, and Barger (7), in which the inert gas method was used to determine intrarenal hemodynamics, represent the most recent evaluations of the influence of ADH on flow distribution within the kidney. They clearly demonstrated effects of physiological amounts of ADH, when infused into the diabetes insipidus dog, on intrarenal blood flow distribution. However, in view of the criticisms which have been advanced against inert gas methods (11), we felt it was necessary to evaluate the influence of ADH on intrarenal hemodynamics utilizing a different technique. Since microspheres appear to be reasonable indicators of cortical flow distribution (11), our analysis is based on this approach.

In the present studies, we have evaluated the influence of physiological amounts of ADH on intrarenal blood flow distribution in acutely hypothysectomized (HPX) dogs and in female rats with hereditary diabetes insipidus rats (Brattleboro strain).

Methods. Dog studies. Twelve mongrel dogs of either sex (11–16 kg) were anesthetized with pentobarbitol and ventilated with a Harvard apparatus respirator. Hypophysectomy was accomplished via a transbuccal approach similar to that used by Fisher *et al.* (8). In addition, saline containing para-aminohippurate (PAH) and creatinine was infused at 0.05 ml/min via a PE100 catheter into the right external jugular vein. In order to balance urinary fluid loss, appropriate amounts of saline were infused via a second PE100 catheter in the right jugular vein. The left femoral artery was cannulated with PE100 tubing and served for monitoring blood pressure (Physiograph recorder and transducer). A second PE100 catheter was advanced into the thoracic aorta via the right femoral artery for collection of arterial samples and injection of labeled microspheres (see below for details). Ureters were cannulated with PE100 tubing, and Kifa catheters (U.S. Catheter Corp.) were positioned in the renal veins via the femoral veins.

After hypophysectomy, urine osmolarities were followed until a steady state was obtained. Three dogs failed to produce a urine below 300 mOsm/liter and were not included in the study. After urine osmolarities had stabilized (approx 2 hr), two consecutive clearance periods were performed with midpoint arterial and renal venous samples. ^{169}Yb-labeled microspheres (15 ± 5 μm, 3M Corp.) were rapidly injected into the thoracic aorta with 3 ml of saline. An ADH infusion (0.6 mUADH/min/kg body wt) was then initiated, and after 60–90 min a second clearance period was performed as above, except that ^{85}Sr-labeled spheres were injected (15 ± 5 μm, 3M Corp.).

Rat studies. Female Brattleboro rats were anesthetized with Inactin (0.1 mg ip/g body wt) and tracheotomized with PE240 tubing. Stretched PE50 catheters were placed in the left femoral vein and artery. The arterial cannula was advanced into the thoracic aorta and used for blood pressure recordings, arterial sampling, and microsphere injections. In 4 of the 11 rats utilized, it was necessary to cannulate the right carotid artery as the femoral catheter could not be advanced into the thoracic aorta. Two milliliters of a hypotonic solution (75 mM NaCl) containing [^{3}H]inulin (New England Nuclear) + unlabeled inulin and PAH were infused into the external jugular vein via a PE50 catheter during a 10-min period. A sustaining infusion of the above solution was then started at 0.05 ml/min. When urinary osmolarities had stabilized, a control clear-

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ance period was performed and ^{169}Yb-labeled microspheres were injected. ADH was then administered iv at 0.1 mU ADH/min/kg body wt for at least 1 hr (average time = 1.5 hr). This was followed by a second clearance period and injection of ^{85}Sr-labeled microspheres.

At the termination of both dog and rat experiments, the kidneys were removed, weighed, and placed in 10% formaldehyde overnight. Two cortical zones, an outer and inner cortex, were easily discernible in both species following this fixation. The kidneys were decapsulated and with the exception of the superior and inferior poles, virtually the entire outer cortex was removed, weighed, and counted. The small amount of outer cortex remaining on the renal sections was then removed and the entire inner cortex was separated, weighed, and counted. All remaining pieces were also counted for quantitation of total renal counts of each microsphere nuclide. Radioactivity was determined on a Packard gamma scintillation counter (Model 3002).

Sodium and potassium concentrations were determined on an Advanced Instrument Corp. flame photometer. Creatinine was estimated by the method of Folin and Wu (9), and PAH by the diazotization procedure of Bratton and Marshal (4). [^{3}H]inulin radioactivity was determined on a Beckman liquid scintillation counter (Model LS 333). Urine osmolarities were measured by freezing point depression (Precision Osmette).

In the dog studies, renal blood flow (RBF) calculations were based on the renal extraction and clearance data for PAH. Blood flow per gram inner and outer cortex were calculated according to the method of Katz *et al.* (11). Since renal PAH extractions were not measured in the rat, regional blood flows to outer and inner cortices could not be estimated in those studies. All statistical analyses were based on the Student's *t* test for paired data.

Results. Intrarenal blood flow distribution in the HPX dog. The ratio of inner to outer cortical blood flow (IC/OC ratio) in hypophysectomized dogs before and during infusion ADH is shown in Fig. 1. Paired Student's *t* test analysis showed a significant decrease in the IC/OC ratio during infusion of ADH into the HPX animal ($P < 0.01$).

The decrease observed in the IC/OC ratio following ADH shown in Fig. 1 could be due to an increase in outer cortical flow or a decrease in inner cortical flow. In order to ascertain which factor predominated, inner and outer cortical blood flow rates were compared before and during ADH infusion (Fig. 2). As may be seen in Fig. 2, there was a significant decrease in inner cortical flow with ADH ($P < 0.01$), whereas outer cortical flow showed no consistent change. Total renal blood flow, estimated from PAH clearance and extraction data, did not change significantly with ADH infusion ($P < 0.01$). These data are shown in Fig. 3,

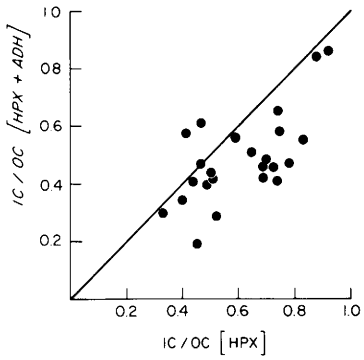

FIG. 1. The ratio of inner to outer cortical blood flow (IC/OC ratio) in 12 hypophysectomized dogs is plotted before and during infusion of 0.6 mU ADH/min/kg body wt (solid points). In all figures, the line of identity is shown.

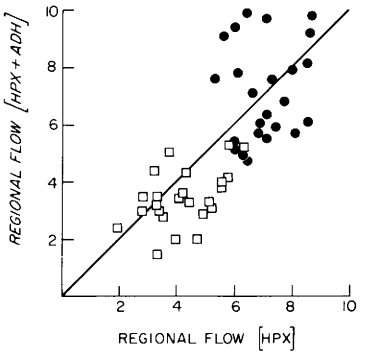

FIG. 2. Inner cortical blood flow (squares) and outer cortical blood flow (points) are plotted before and during infusion of 0.6 mU ADH/min/kg body wt into hypophysectomized dogs. Values are expressed as milliliter per minute per gram of inner or outer cortex.

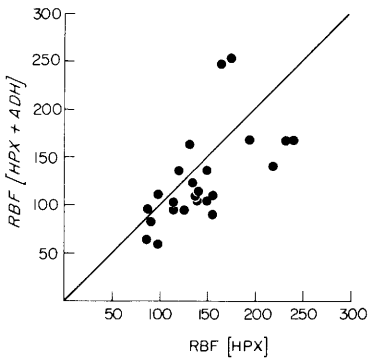

FIG. 3. Renal blood flow in milliliters per minute is plotted before and during infusion of ADH in HPX dogs.

which is a plot of renal blood flow before and during ADH infusion in the HPX dog.

Influence of ADH on solute excretion and GFR in the dog. Urine osmolarities averaged 185 mOsm/liter before ADH and 557 mOsm/liter during infusion of ADH into HPX dogs. Paired Student's t test analysis showed that the increase in urine osmolarity with ADH was significant at $P < 0.01$.

Increases in Na and K excretion rates were consistently observed following ADH infusion. Na excretion averaged 2.4 μequiv/min before ADH and 31 μequiv/min during ADH infusion. Potassium excretion averaged 6.24 μequiv/min after hypophysectomy and 19.6 μequiv/min following ADH. Paired Student's t test analysis showed that the increase in both Na and K excretion with ADH were significant at $P < 0.05$.

Infusion of ADH into the HPX dog was accompanied by a small but, nonetheless significant, increase in glomerular filtration rate ($P < 0.05$). GFR averaged 0.54 ml/min/g kidney weight before ADH and 0.58 ml/min/g kidney weight during ADH infusion.

Rat Studies. Aspects of the influence of ADH on intrarenal hemodynamics in the Brattleboro rat were similar to those described in the HPX dog. Of particular interest is the fact that the IC/OC ratio was significantly lower after ADH infusions into hypotonically expanded Brattleboro rats ($P < 0.01$). These data are graphically illustrated in Fig. 4, which shows the ratio of inner to outer cortical blood flow before and after ADH infusion. It is also of interest to note that the clearance of PAH (C_{PAH}) did not change significantly during ADH infusion. Thus, C_{PAH} averaged 1.49 ml/min/g kidney weight before and 1.35 ml/min/g kidney weight during ADH infusion.

In contrast to the dog studies, ADH infusion into Brattleboro rats produced no consistent changes in [^{3}H]inulin clearance or electrolyte excretion rates. Sodium excretion rates averaged 3.5 μequiv/min before ADH and 2.9 μequiv/min during ADH infusion. Similarly, K excretion averaged 1.7 μequiv/min before and 1.8 μequiv/min after ADH. Mean urinary osmolarities were 601 mOsm/liter before ADH and 964 mOsm/liter during ADH infusion.

Discussion. The data presented in this paper clearly show that physiological amounts of ADH infused into both diabetes insipidus dogs and rats influences intrarenal blood flow distribution. Thus, in the presence of ADH the ratio of inner cortical blood flow to outer cortical flow is lower in both species (Fig. 1 and 4). In the HPX dog, the decrease in the IC/OC ratio observed following ADH is a reflection of a decrease in inner cortical blood flow (Fig. 2). Since PAH extractions were not measured in the rat studies, no firm conclusion can be drawn concerning absolute changes in regional blood flow following ADH. However, the constancy of other renal parameters in the rat before and after ADH, such as GFR, C_{PAH}, and electrolyte excretion suggest that ADH also re-

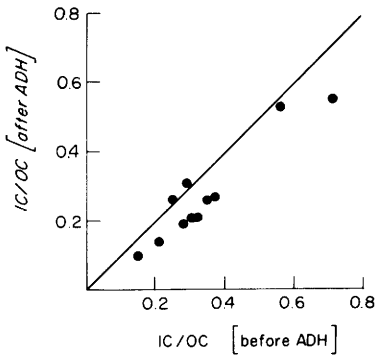

FIG. 4. The ratio of inner to outer cortical blood flow (the IC/OC ratio) is shown before and during infusion of 0.1 mU ADH/min/kg body wt into 11 Brattleboro rats. Values represent the mean ratio of right and left kidney in each study.

sulted in a decrease in inner cortical blood flow in this species.

The influence of ADH on juxtamedullary flow discussed above differs from the results of Fisher *et al.* (8), who used inert gas washout analysis to estimate intrarenal blood flow distribution. They described a decrease in juxtamedullary flow as ADH levels fall after hypophysectomy. The reason for the difference between the present results and those of Fisher *et al.* is not readily apparent, but may relate to the inherent problems of inert gas data interpretations (11).

Infusion of ADH into HPX dogs resulted in a natriuresis, kaliuresis, and a small but significant increase in glomerular filtration rate. While it is tempting to speculate that the enhanced GFR observed with ADH accounts for the increase in Na and K excretion, Humphreys *et al.* (6) have shown that changes in GFR cannot account for ADH-induced natriuresis. Thus, other factors would appear to be involved in this natriuretic response to ADH.

In contrast to the dog studies, we observed no natriuretic or kaliuretic response to ADH in the rat. Atherton *et al.* (1) have shown a marked natriuretic response to 0.6 mU ADH/min/kg body wt in rats undergoing water diuresis. However, only a small natriuretic response was observed with 0.05 to 0.15 mU ADH/min/kg body wt. Furthermore, they found that the natriuretic response reached a maximum 1 hr after initiating the infusion of ADH. The fact that we used a relatively low dose of ADH in our rat studies (0.1 mU/min/kg body wt) and waited an average of 1.5 hr before clearance periods were performed probably accounts for the difference between our results and those of Atherton *et al.* (1).

One of the important aspects of our data relates to the effect of ADH on medullary blood flow. Since the medullary vasculature derives predominantly from juxtamedullary glomeruli (3), it seems probable that the decrease in juxtamedullary blood flow with ADH is accompanied by a decrease in medullary flow. There have, however, been conflicting reports concerning the influence of ADH on medullary blood flow. Thurau *et al.* (14) showed that mean transit times of Evans blue through the inner medulla of dogs increased after pharmacological doses of ADH were administered to dogs in water diuresis. However, Kramer and associates (see Thurau, 1964), using a similar mean transit time approach, could not demonstrate effects of physiological amounts of ADH on medullary hemodynamics. Similarly, Aukland (2) was unable to demonstrate effects of physiological doses of ADH on medullary flow using hydrogen gas washout techniques. Finally, Fourman and Kennedy (10), using fluorescent dye, and Cross *et al.* (5), using timed uptake of ^{131}I-labeled serum albumin, demonstrated an effect of ADH on medullary flow, whereas Finberg and Pearl (7), using the carbon injection approach, found no changes with ADH.

The importance of medullary blood flow on the osmotic gradient in the medulla has been emphasized by Stephenson *et al.* (12). If ADH does indeed decrease vasa recta blood flow in the dog and the rat, as the data in the present paper strongly suggest, such an effect would clearly be advantageous in the production of a concentrated urine.

Summary. The distribution of labeled microspheres within the renal cortex was used to evaluate the influence of physiological amounts of antidiuretic hormone on intrarenal blood flow distribution in hypophysectomized dogs and in rats with hereditary diabetes insipidus. In both species, intravenous infusions of ADH caused a significant decrease in the ratio of inner to outer cortical blood flow. The change in blood flow distribution observed in the hypophysectomized dog with ADH was primarily a consequence of a decrease in inner cortical blood flow. No consistent changes in outer cortical blood flow were found. Also in the dog, glomerular filtration rates and electrolyte excretion rates (Na and K) increased following ADH. In contrast, ADH infusion into Brattleboro rats caused no change in glomerular filtration rate or excretion of Na and K.

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