Influence of Dietary Sodium on the Renal Isoforms of 11β-Hydroxysteroid Dehydrogenase (44101)

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Abstract. **Endogenous glucocorticoids are converted to their biologically inert 11-dehydroderivatives by isoforms of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). The low-K_m, NAD^+-dependent renal isoform (Type 2) identified in the distal nephron protects mineralocorticoid receptors from activation by endogenous glucocorticoids. The function of high-K_m, $NADP^+$-dependent renal isoform (Type 1) is less well understood. Since glucocorticoids may modulate sodium transport in renal proximal tubules (PT), we hypothesized that Type 1 activity in this segment may be regulated by dietary Na^+. 11β-HSD activity was assessed in homogenates of canine PT by the conversion of cortisol to cortisone in the presence of $NADP^+$ 200 μ*M*. A high-Na^+ diet for 4 days increased the V_{max} 4-fold, with no change in the Type 1 K_m (40 mEq/day Na^+ diet: K_m 0.959 μ*M*, V_{max} 3.40 pmoles/min/mg protein versus 150 mEq/day Na^+ diet: K_m 0.962 μ*M*, V_{max} 14.8 pmoles/min/mg protein). Type 1 mRNA also rose in the salt repleted animals. The high-Na^+ diet produced no detectable change in the Type 2 isoform enzyme kinetics and mRNA level. No reverse oxo-reductase activity was noted with either renal isoform. Thus, renal Type 1 11β-HSD can be regulated by dietary Na^+ independent of changes in the renal Type 2 isoform.** [P.S.E.B.M. 1997, Vol 214]

Thus far, two isoforms of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) have been identified in the mammalian kidney. Both isoforms inactivate endogenous glucocorticoids, transforming them into their biologically inert 11-dehydro derivatives (1–3). The proximal tubule, specifically the S2 and S3 segments, contains the Type 1 isoform of the enzyme (1, 4). This isoform has an apparent K_m of approximately 10^{-6} *M*, and based on the present and other available data it may be able to utilize both NAD^+ and $NADP^+$ as a co-factor (5). Interestingly, its expression and activity vary considerably among species (6). In the human (6, 7), the sheep (8), and now the dog, Type 1 isoform activity is quite low, while in the mature rat it is highly expressed and very active (9). The activity in the rat, however, changes with development; it is barely measurable at birth and increases to near adult levels by the second week of life (4). The physiologic function of this enzyme in proximal tubular segments remains to be defined; this is especially true given the extreme variation in the enzyme activity among different species. There are several observations indicating that glucocorticoids may, under certain circumstances, play a significant role in proximal tubule ion transport. Glucocorticoids increase the activity of the Na^+/H^+ antiporter (NHE-3) present along the apical membrane of proximal tubules (10–11) and also appear to upregulate Na, K-ATPase activity in parallel with a rise in mRNA for both the α and β subunits (12). Moreover, we have recently shown that, when 11β-HSD in cultured proximal tubule epithelial cells is inhibited, corticosterone significantly enhances angiotensin II–stimulated sodium transport (13).

Renal cortical collecting duct cells express a different isoform of 11β-HSD (Type 2) (2). This isoform has

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an apparent K_m of approximately 10^{-8} M and a clear preference for NAD^+ as a co-factor. Its biologic role is to limit the access of endogenous circulating glucocorticoids to mineralocorticoid receptors present in these renal epithelial cells (3).

The present studies were designed to determine whether exposure to a high-sodium diet would change the expression and/or activity of either Type 1 or Type 2 isoforms of 11β-HSD. A high-sodium diet was chosen because this diet presumably eliminates any need for any additional hormonally induced renal sodium reabsorption. The present studies show that the renal Type 1 isoform is induced with a high-sodium diet, with no detectable changes in the Type 2 isoform. Moreover, these studies suggest that the two renal isoforms of this enzyme can be regulated by different mechanisms.

Materials and Methods

Ten female mongrel dogs (18.2–20.5 kg body wt, 9–10 months of age) were used for these studies. Five dogs were in the experimental group, and five dogs served as controls. The dogs were kept in an environmentally regulated indoor pen with free access to food and water for 2 weeks prior to initiating any experimental protocols. An additional male control dog was included in the mRNA studies for purposes of gender comparison. Once the high-salt diet was initiated, food amounts were regulated, but the dogs were given free access to water. A high-sodium diet was administered daily for a period of 5 days to the five dogs in the experimental group (14–16). The high-sodium diet consisted of two cans of H/D Diet dog food (Prescription Diet, Topeka, KS) with 75 mEq of NaCl added per can. The animals designated controls were given two cans of the identical regular-formulated dog food which mirrored the ionic and nutritional content except for sodium. No animals failed to finish their meals. Water consumption was not monitored except to note that no control dog gained more than 0.20 kg during the study period. The experimental animals did not demonstrate a significant change in body weight during the 5 days of a high sodium diet. The weight gain observed in experimental animals was not statistically different from that seen in control animals. No blood pressures were obtained in any of the animals but from previously reported studies following the same protocol, mean arterial pressures do rise in dogs within 24 hr of sodium loading (14, 15). All animals were fasted for 12 hr prior to sacrifice. Thiopental sodium was given in a dose sufficient to induce anesthesia. Using a midline abdominal incision, the renal pedicles were clamped, and the kidneys removed and immediately placed in ice. A cortical wedge was taken and flash frozen in liquid nitrogen. The kidneys were then perfused with previously aerated (95% O_2/5% CO_2) Krebs-Henseleit bicarbonate buffer (KHB) to flush the organs of residual blood. The kidneys were sliced sagitally, and the cortex removed and kept in ice-cold KHB during the isolation procedure for proximal tubule segments.

Purified canine proximal tubule segments were isolated from renal cortex as previously outlined (17, 18). The cortex was sliced with a Stadie-Riggs microtome and then incubated in 21 ml of aerated KHB containing collagenase (1.43 mg/ml) and bovine serum albumin (5 mg/ml) for 45 min. Following the incubation, the incubation medium was filtered and the filtrate serially centrifuged through a Percoll density gradient (Pharmacia Biotech LKB, Uppsala, Sweden). Segments of nearly pure (96%) S_1 and S_2 kidney tubules, as judged by microscopic and biochemical methods, were recovered from the Percoll gradient (18), snap frozen in liquid nitrogen and stored at −70°C until studied. Tubules were stored frozen for no longer than 2 months prior to study. All studies were conducted on pooled tissue samples from the experimental and control groups.

Metabolism Studies. Tritiated cortisol (sp act: 53.0 Ci/mmole) was obtained from New England Nuclear (Boston, MA). Nonradioactive cortisol and other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

For metabolism experiments, pooled homogenates of purified canine renal proximal tubules or canine renal cortex were prepared in sodium-phosphate-sucrose buffer (10 mM sodium phosphate, 0.25 M sucrose) at pH 7.4 and 290 mOsm/kg in the presence of the protease inhibitors leupeptin and aprotinin (0.001 mg/ml for each). The cofactor $NADP^+$ 200 μM was added to assay Type 1 11β-HSD activity. When Type 2 11β-HSD activity was being assessed, NAD^+ 10 μM was added to the homogenate made from renal cortex. This concentration is approximately two times the observed co-factor K_m for this isoform and was chosen to optimize the separation of the two isoforms. From previous studies, the apparent K_m NAD^+ for Type 2 was calculated to be 2.9 μM in cultured rabbit cortical collecting duct cells (2) and was 5.3 μM in cultured MDBK cells, a model of the distal tubule (Brem, unpublished observations). All samples were incubated at 37°C. Cortisol was used as the substrate in these studies in concentrations ranging from 10^{-8} to 10^{-6} M. Tracer label (1 μCi/ml) was added to the reaction medium. In the kinetics and time course experiments, each data point represents the mean of at least three separate observations from pooled samples of tubules or renal cortex.

At the completion of an incubation, the reaction was stopped by adding 1 ml of methanol to each sample, and the homogenates were centrifuged at 3600 rpm for 2 min. The steroids present in the supernatant were separated by HPLC using a Dupont Zorbax C8 column eluted at 55°C at a flow rate of 1 ml/min using 38% methanol for 30 min and increasing linearly up to 100%

for an additional 20 min. The various steroid compounds were observed by monitoring radioactivity on line with a Packard Radiomatic Flo-One/Beta Series A-500 counter connected to a Dell Optiplex 425 S/L computer running Microsoft Windows 3.1 and A505 Flo-One for Windows (version 2.0A) and were identified by comparing the retention times to those of known standards. Results were normalized to the sample protein concentration; the mean protein concentration was 0.98 ± 0.21 mg/ml (mean ± SEM) (Bradford protein assay; Bio-Rad Laboratories, Richmond, CA). Enzyme kinetics (K_m and V_{max}) were calculated from a double reciprocal plot (Lineweaver-Burk plot) drawn using Cricket Graph v1.2.3 with a line of best fit determined from the data.

RNA Studies. RNA was extracted from previously frozen (−70°C) renal cortex isolated from salt-loaded and control animals using RNAzol B (CINNA/BIOTECX, Houston, TX). The RNA was mixed with chloroform (1 volume homogenate/0.1 volume chloroform), then cooled to 4°C for 5 minutes and centrifuged at 12,000*g* for 15 min at 4°C. The aqueous phase was removed and mixed with an equal volume of isopropanol, allowed to cool to 4°C for 15 min and then centrifuged at 12,000*g* for 15 min. The supernatant was removed and the RNA pellet mixed with 75% ethanol (0.8 ml/50–100 μg RNA) and centrifuged at 7500*g* for 8 min. Finally, the RNA pellet was dried under vacuum for 10–15 min, solubilized in DEPC-treated water with 1 m*M* EDTA at pH 7 and stored at −70°C until further use. The cDNA probe for human Type 1 11β-HSD was a gift from Dr. Perrin White (Dallas, TX) (7), while the cDNA for human Type 2 11β-HSD was kindly provided by Dr. Zygmunt Krozowski (Prahran, Australia) (19). Human Type 2 11β-HSD has only 14% identity with the Type 1 enzyme isoform (19). The cDNA probe for G3PDH (glyceraldehyde-3-phosphate-dehydrogenase) served as a reference (Clontech, Palo Alto, CA). Northern blots were prepared by using 10–20 μg of total RNA/lane in a 1% agarose gel with a borate buffer and 2% formaldehyde. Electrophoresis was performed using a ProbeTech II (Oncor, Gaithersburg, MD) electrophoresis/vacuum blotting device. Following electrophoresis, the gel was transferred to a SureBlot nylon membrane (Oncor) in 10× SSC using a vacuum. After being baked at 80°C, blots were prehybridized in 45% deionized formamide with 10× Denhardt's solution, sheared salmon sperm DNA, and dextran sulfate at 42°C. 11β-HSD and G3PDH gene-specified cDNA probes were labeled with ^{32}P dCTP using a random primed labeling kit (Boehringer Mannheim, Germany), heat denatured, and added to the hybridization reaction. Blots were washed at low stringency in 0.1× SSC at 50°C prior to autoradiography. Quantitation of Northern blots was done using a Microtek 600zs flatbed scanner connected to an Apple Quadra 650 computer running Macintosh System 7.1 and NIH Image version 1.52.

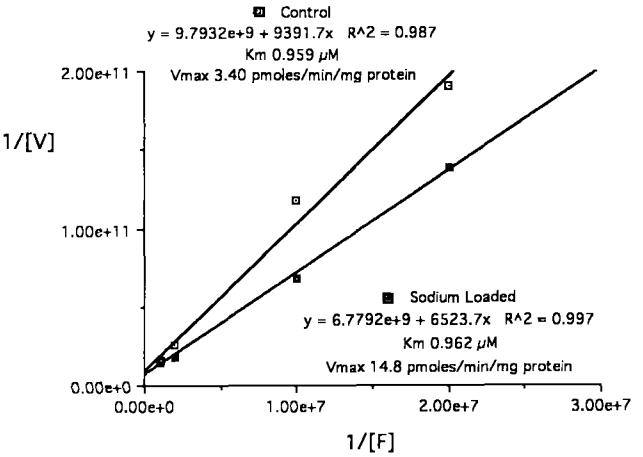

Figure 1. 11β-HSD Type 1 enzyme kinetics (Lineweaver-Burk plot) in homogenates prepared from canine renal proximal tubules harvested from control or sodium replete animals. $NADP^+$ 200 μ*M* was used as a co-factor with concentrations of cortisol ranging from 10^{-5} *M* to 10^{-8} *M*. Incubations were conducted for 10 min.

Samples were analyzed without prior knowledge of the treatment protocol.

Results

Kinetic Studies. To assess the enzymatic conversion of cortisol to cortisone as a function of time, homogenates of proximal tubules were prepared and incubated with 10^{-8} *M* cortisol for periods up to 90 min. The reactions were conducted in the presence of 200 μ*M* $NADP^+$. The conversion of cortisol to cortisone was nearly linear for periods up to 30 min. When similar experiments were conducted using homogenates of renal cortex, a similar linear increase in the formation of cortisone from cortisol was noted during the first 30 min of the reaction. In separate studies, homogenates prepared from proximal tubular segments were able to utilize NAD^+ and $NADP^+$ equally well as co-factors.

Since 11β-HSD present in proximal tubules appeared to produce a linear increase in the generation of cortisone through 30 min, all the remaining kinetics experiments conducted on homogenates prepared from either proximal tubules or renal cortex were performed after a 10-min incubation period. Proximal tubules harvested from control animals demonstrated an apparent K_m for cortisol of 0.959 μ*M* with a corresponding V_{max} of 3.4 pmoles/min/mg protein. In contrast, proximal tubules harvested from animals previously fed a high-sodium diet had an apparent K_m for cortisol of 0.962 μ*M* with a V_{max} of 14.8 pmoles/min/mg protein, approximately a 4-fold increase in V_{max} (Fig. 1).

In separate studies, homogenates of proximal tubules were incubated with cortisone 10^{-8} *M* and NADPH 200 μ*M* for periods of 30, 60, and 90 min respectively. No oxo-reductase activity (formation of cortisol) was noted after any of the observation periods.

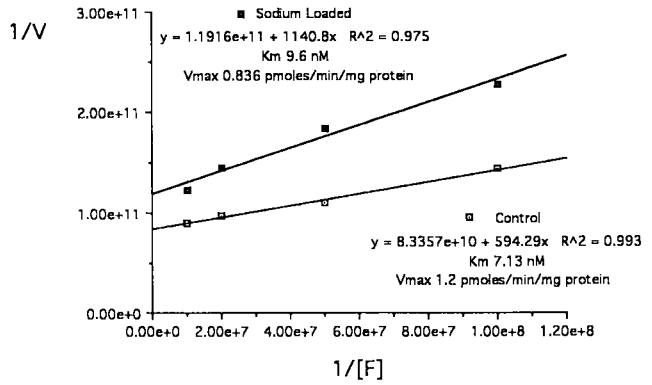

Figure 2. 11β-HSD Type 2 enzyme kinetics (Lineweaver-Burk plot) in homogenates prepared from canine renal cortex harvested from control or sodium repleted animals. NAD^+ 10 μ*M* was used as a co-factor, with concentrations of cortisol ranging from 10^{-7} to 10^{-8} *M*. Incubations were conducted for 10 min.

For purposes of comparison, experiments were done with cortisol and $NADP^+$ in the same concentrations; approximately 40% of the cortisol was converted to cortisone within 90 min.

Kinetic studies were also conducted on 11β-HSD Type 2 in homogenates prepared from renal cortex. In this circumstance, incubations were conducted with 10 μ*M* NAD^+ as a co-factor. This concentration of NAD^+ is approximately 2- to 3-fold the observed K_m for co-factor in the Type 2 isoform present in cortical collecting duct cells (2) and is well below the optimum co-factor requirements for the Type 1 isoform. Homogenates of renal cortex harvested from control animals demonstrated an apparent K_m for cortisol 7.10 n*M* with a corresponding V_{max} of 1.20 pmoles/min/mg protein. In similarly prepared homogenates from animals eating a high-sodium diet, the apparent K_m for cortisol was 9.60 n*M* with a V_{max} of 0.84 pmoles/min/mg protein. Thus, a high-sodium diet had little effect on the V_{max} of Type 2 isoform present in the kidney (Fig. 2).

Studies on 11β-HSD mRNA Expression. Total RNA was isolated from renal cortex of control animals and animals previously fed a high-sodium diet. When the Northern blots were probed with the Type 1 11β-HSD cDNA, an approximately 2-fold increase in specific mRNA (relative to GAPDH expression on the same blot) was noted in animals that had been previously fed a high-sodium diet (Fig. 3). These changes in 11β-HSD Type 1 correlated well with the observed increase in V_{max}. No detectable change in mRNA level for the Type 2 11β-HSD was observed, however.

Discussion

The present studies provide indirect evidence that there is an increase in 11β-HSD Type 1 in renal proximal tubules and that this increase is induced by a high-sodium diet. We did attempt to confirm the observed rise in enzyme activity (V_{max}) and in enzyme message with Western blot analysis. However, the available polyclonal antibody raised against rat hepatic 11β-HSD was not specific and produced Western blots with numerous bands in preparations made both from rat and from dog.

In earlier studies, performed in the newborn rat kidney, we observed that the activity of 11β-HSD Type 1 changes during development. Little or no Type 1 activity was noted at birth, but the expression of this isoform and its activity increased markedly by the tenth day of life (4, 9). These studies, taken with the present experiments, are consistent with the view that the Type 1 isoform of 11β-HSD present in proximal tubular segments is capable of being induced. The concept that this isoform can vary in its expression and activity is also compatible with the finding that different species demonstrate wide variances in Type 1 activity in renal proximal tubular segments (6, 20), possibly reflecting variations in development and/or diet.

Our studies are the first to clearly support the view that the two renal isoforms of 11β-HSD are regulated by different effector mechanisms. Factors that increase the expression and activity of the Type 1 isoform in proximal tubules did not produce an obvious detectable change in the Type 2 expression or activity. Such differential regulation may have physiologic consequences. A high-sodium diet leads to expansion of the extracellular fluid space and is normally associated with enhanced urinary sodium excretion. Under these conditions, enhanced local glucocorticoid metabolism (inactivation) would prevent glucocorticoid binding to glucocorticoid receptors in proximal tubular segments and indirectly may prevent excess glucocorticoid from binding to min-

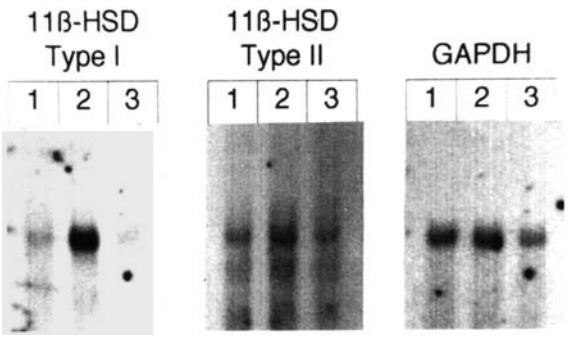

Figure 3. Northern blot of total RNA isolated from canine renal cortex of control and sodium repleted animals. The blot was probed for 11β-HSD Type 1 and 2 as well as GADPH. GADPH was used as a reference for sample loading and RNA integrity. Lanes 1 and 3 are from control female and control male dogs, respectively. Enzyme message for Type 1 11β-HSD is clearly increased in canine kidneys from sodium repleted animals (Lane 2). Sodium loading produced no detectable change in the Type 2 11β-HSD message from sodium repleted animals (Lane 2) compared with controls (Lanes 1 and 3). Densitometry readings for Lanes 1–3 were 11β-HSD-1/GADPH: 0.66, 1.22, 0.72; and 11β-HSD-2/GADPH: 0.60, 0.69, 0.53. The calculated sizes of the three mRNA species are: Type 1 11β-HSD 1700 nuc., Type 2 11β-HSD 1200 nuc., and GAPDH 1650 nuc. Minor lower–molecular weight cross-hybridizing bands were present in the blot probed with the heterologous human 11β-HSD2 cDNA probe.

eralocorticoid receptors in the cortical collecting ducts (1). Moreover, the 11-dehydroderivatives generated in the proximal tubule are released into the blood or urinary space and may gain access to cortical collecting duct cells. This may be of some significance since the 11-dehydroderivatives of endogenous glucocorticoids have been shown to inhibit aldosterone-induced sodium transport (21–22). Thus, glucocorticoid-induced sodium transport would be blunted, and the antinatriuretic effects of any circulating aldosterone would also be suppressed.

Rat renal proximal tubules normally express a high quantity of Type 1 11β-HSD mRNA and activity (9), in direct contrast to the findings in the dog. In preliminary studies, King and Souness (unpublished observations) have noted that Sprague-Dawley rats chronically fed a low-sodium diet show a decrease in Type 1 11β-HSD message consistent with transcriptional regulation of the enzyme. These observations taken together with the present studies are strong evidence in favor of the fact that changes in dietary sodium can over time affect the synthesis and net activity of renal Type 1 11β-HSD. The dietary effects on 11β-HSD may be either direct or mediated indirectly through blood pressure or hormonal factors.

The Type 1 11β-HSD in renal proximal tubules appears to be regulated in another way. The Type 1 isoform found in liver (23) and vascular smooth muscle (24) is a bidirectional enzyme capable of both the forward dehydrogenase and reverse oxo-reductase activity. In contrast to our findings in vascular smooth muscle cell homogenates, where we easily demonstrated bidirectional enzyme activity, we have only been able to show dehydrogenase activity in identically prepared homogenates of canine proximal tubules (present studies) and in cultured proximal tubule renal cell lines containing the Type 1 isoform (LLCPK-1 cells and IRPT cells derived from rat proximal tubule; Brem, unpublished observations). Post transcriptional modification and/or the redox state of the available cofactor may account for these differences in Type 1 enzyme action.

Regulation of the Type 2 isoform of 11β-HSD located in cortical collecting duct cells is likely to be somewhat more complex. The physiologic role of this isoform is to serve as "a guardian" over the mineralocorticoid receptor, preventing glucocorticoids from directly binding to that receptor and inducing a mineralocorticoid-like response (1, 3). Stable concentrations of Type 2 isoform of the enzyme are required to ensure mineralocorticoid receptor specificity. From our findings, it appears as though renal 11β-HSD Type 1 is inducible under certain specific conditions, while 11β-HSD Type 2 is regulated by completely different processes or is a constitutive enzyme consistent with its stated function. The processes involved with the induction of 11β-HSD Type 1 are unknown at this point. From preliminary studies, prime candidates include those factors involved with growth and/or maturation and factors which are involved with the induction of a natriuresis following extracellular fluid volume expansion.

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