Comparison of ATPase in Right and Left Kidneys of the Rat[1] (37545)

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The uneven distribution of Na K ATPase along the renal tubule of the rat has only recently been recognized (1). The resulting unequal distribution of this enzyme in the cortex and medulla (2) is currently an important consideration when renal Na K ATPase activity is assayed (3). Usually, this enzyme is measured in one kidney or in tissue pooled from both kidneys of the experimental animal. To the extent that equal quantities of tissue from each kidney are included in the measurement, the resultant enzyme activity approaches the mean of the enzyme activity of the two kidneys. In fact, equal quantities of tissue from each kidney are rarely obtained, since it is commonly assumed that the specific activity of Na K ATPase is equal in both kidneys. This study was undertaken to test this assumption.

Methods. Male albino Sprague–Dawley rats weighing 180–300 g were maintained on a standard Purina Rat Chow diet with free access to water. Under light ether anesthesia, right and left kidneys were removed in random order and placed in chilled 0.9% saline. After removal of the capsule, each kidney was weighed. Sagittal hemisection of the kidney was performed revealing the boundary between outer (red) medulla and cortex. Samples of each zone were obtained by careful dissection of this boundary. The inner (white) medulla was discarded. Dissection was performed on a filter paper moistened with saline and placed upon a glass petri dish resting on ice. The specific activity of Na K ATPase in whole homogenates of renal cortex and outer medulla of each kidney was determined as previously described (3). A minimum of 20 min after homogenization samples were incubated in a medium at pH 7.8 containing NaCl, 100 mM; KCl, 20 mM, imidazole buffer, 10 mM; $MgCl_2$, 6 mM; and disodium ATP (Sigma Chemical Corp., Grade II), 6 mM. The reaction was begun by addition of $MgCl_2$ and ATP and carried out at 37° for 15 min in a shaking water bath. Addition of 1 ml of ice-cold 35% (w/v) trichloroacetic acid terminated the reaction. The samples were then centrifuged, and determination of inorganic phosphate was performed on the supernatant by the method of Fiske and Subbarow (4). Optical density was read at 660 nm in a Gilford spectrophotometer. Na K ATPase activity was defined as the difference between the inorganic phosphate released in the presence and in the absence of potassium in the incubation mixture. The protein content of the whole homogenate was determined by the method of Lowry *et al.* (5). Results were expressed as micromoles of inorganic phosphate per milligram of protein per hour, μmoles P_i/mg protein/hr. The differences in the specific activity of Na K ATPase activity between left and right kidneys of the same animal were compared to zero using the paired t test. All statistical tests were two-tailed and the null hypothesis was rejected at a p value of < 0.05.

Results. The values for the wet weight, and the specific activity of Na K ATPase and Mg ATPase of left and right kidneys are presented in Table I. There was no significant difference in wet weight or the specific activity of Na K ATPase or Mg ATPase when all left kidneys were compared to all

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TABLE I. Weight and ATPase Activity of Right and Left Kidneys.

		Na K ATPase (μmoles P_i/mg protein/hr)[a]		Mg ATPase (μmoles P_i/mg protein/hr)	
	Wet wt (g)[a]	Cortex	Medulla	Cortex	Medulla
Left kidney	0.95 ± 0.04 (18)	12.12 ± 0.30 (18)	23.96 ± 0.81 (16)	43.60 ± 0.71 (18)	48.11 ± 1.14 (16)
Right kidney	0.96 ± 0.04 (18)	12.29 ± 0.39 (18)	21.64 ± 0.99 (16)	43.65 ± 0.56 (18)	46.22 ± 1.25 (16)
Differences[b]	−0.01 ± 0.01 (18)	−0.18 ± 0.40 (18)	2.32 ± 1.01 (16)	−0.04 ± 0.52 (18)	1.89 ± 1.36 (16)
p[c]	NS	NS	<.05	NS	NS

[a] All values are mean ± standard error (n).
[b] Mean of the differences between the left and right kidney of the same animal.
[c] p value for two-tailed t test of the differences.

right kidneys. When the differences in enzyme activity between the left and right kidneys of the same animal were compared, however, a significant difference was apparent. The specific activity of Na K ATPase in the medulla of the left kidney was greater than in the right kidney in 13 of 16 animals. The mean difference between kidneys was 2.32 ± 1.01 μmoles P_i/mg protein/hr, $p < 0.05$, or approximately 11%. There was no difference between left and right kidneys of the same animal when the specific activity of Mg ATPase or cortical Na K ATPase was considered.

Discussion. Our findings indicate that Na K ATPase activity is greater in the outer medulla of the left kidney than in the right kidney of the rat. This asymmetry apparently does not result from the irregular distribution of Na K ATPase along the nephron (1) but could be a consequence of the several anatomical differences between the kidneys. Quite possibly other enzymatic and functional differences are present as well, and if known would be of practical as well as theoretical value.

The small but significant difference in Na K ATPase activity between kidneys not only has physiological implications, but also bears on the design of protocols and preparation of tissue homogenates involving this enzyme assay. Medullary Na K ATPase activity of a single experimental kidney must be as cautiously compared with its contralateral control as with a control derived from one or both kidneys of another animal. Thus, special care is required in the choice of control groups for unilateral nephrectomy and ureteral ligation or diversion studies. The measurement of renal Na K ATPase activity when whole animals are treated and compared, however, would appear reliable as long as tissue from one or both kidneys is consistently included in the sample assayed. We would expect pooled samples to result in less than a 10% difference in medullary Na K ATPase activity between experimental animals solely due to unequal sampling of the two kidneys in each animal.

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