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Regional Differences in the Binding Affinities of β-Adrenergic Receptors in the Canine Kidney Cortex (40661)[1]

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The coupling of β-adrenergic neurotransmitters to membrane-bound receptors results in the activation of cyclic adenosine monophosphate (cAMP) which in turn leads to specific physiologic responses (1). In the mammalian kidney, the stimulation of β-adrenergic receptors may be manifested by increases in renal blood flow (2), sodium and water excretion (3), and/or renin release (4). All of these responses parallel the actions of intrarenally administered cAMP or its analogs (5–7).

Recent studies have suggested a neurogenic role in renal tubular sodium reabsorption (8). Direct contact of adrenergic nerve terminals with basement membranes of proximal and distal tubules has been demonstrated in several species (8, 9). Renal denervation decreases and renal nerve stimulation increases proximal tubular sodium reabsorption (10, 11). Other studies have demonstrated in the dog that intrarenal administration of either isoproterenol or dibutyryl cAMP can decrease proximal tubular sodium reabsorption without changes in renal blood flow (3, 5). Chabardes *et al.* (12) have reported that in the rat β-adrenergic stimulation results in increases in adenylate cyclase activity in segments of the distal tubule, suggesting a further role of the adrenergic system in renal tubular sodium transport.

The kidney is structurally and functionally heterogenous (13). Nephrons from the superficial cortex have been shown to differ from inner cortical nephrons in glomerular size, plasma flow, filtration, and sodium reabsorption (13). The degree of adenylate cyclase activation produced by hormones may vary depending upon the regional location of these nephrons (14). In addition, differences in adrenergic innervation and norepinephrine content in the inner and outer regions of the kidney have been noted (15, 16). Therefore, when studying tubular adrenergic receptors, it becomes important to assess membranes derived from both the inner and outer areas of the kidney cortex.

The advent of highly specific radioligands for direct binding studies of β-adrenergic receptors (17) has made possible the quantification of these receptors in a variety of tissue sources (18–20). Utilizing the radiolabeled β-adrenergic antagonist, [^{3}H]dihydroalprenolol ([^{3}H]DHA), studies were undertaken to characterize β-adrenergic receptors in tubular tissue obtained from two different regions of the canine kidney cortex. The results indicate that β-adrenergic receptors within the renal cortex exhibit distinct regional differences in their affinities for dihydroalprenolol.

Materials and methods. Adult mongrel dogs kept on a standard laboratory diet (Purina Chow) were sacrificed by intravenous administration of sodium pentobarbital. Both kidneys were immediately excised, decapsulated, and placed into an ice-cooled (4°) buffer consisting of 0.25 *M* sucrose, 10 m*M* Tris–Cl, and 1 m*M* EDTA adjusted to a pH of 7.5. The kidneys were sagitally sectioned and the cortex divided into an outer 2/3 and inner 1/3 segments (based on wet weight) by methods previously described from this laboratory (21). Renal plasma membranes from each area were subsequently isolated by differential centrifugation techniques according to the procedure of Fitzpatrick *et al.* (22). The final pellet (containing primarily tubular membranes) was resuspended in an incubation buffer consisting of 75 m*M* Tris–Cl and 10 m*M* $MgCl_2$ (pH 7.5). β-Adrenergic receptors in renal plasma membranes were subsequently assayed using the ligand [^{3}H]DHA according to the method of Lefkowitz (23)

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using a vacuum filtration technique. All incubations were conducted in 150 μl of incubation buffer (final volume) at 23°. The temperature of 23° was chosen because at 37° spontaneous dissociation of the ligand from the membrane occurred within 5 min. Incubation time was 7 min except for the association–dissociation studies.

Association and dissociation kinetics for inner and outer cortical membranes (approximately 1.0 mg protein) were performed using 30 n*M* [^{3}H]DHA. Following a 2-min equilibration period association studies were started by the addition of the ligand to the incubation mixture and assessed at periodic intervals for at least 15 min. Dissociation of the ligand from the receptor was initiated by the addition of 1-propranolol (10^{-4} *M*) to membranes previously incubated with [^{3}H]-DHA for 7 min. The dissociation was assessed at periodic intervals for at least 15 min.

Linearity of ligand binding to renal plasma membranes was assessed in a similar fashion by incubating [^{3}H]DHA (30 n*M*) in the presence of varying concentrations of membrane protein.

[^{3}H]DHA binding curves for each tissue were determined using a fixed amount of membrane protein (approximately 1.5 mg) incubated with varying concentrations of the radioligand. In each experiment, nonspecific binding of [^{3}H]DHA (approximately 20% of the total binding) was determined from concurrent incubations performed in the presence of 1-propranolol (100 μ*M*). Specific binding was assessed as the difference between the total and the nonspecific binding. Receptor occupancies (R_o) and dissociation constants ($-K_d$) were calculated from analyses of Scatchard plots.

Competition experiments were also performed in which varying concentrations of antagonists were added to the incubation mixture as indicated. Each assay was performed in triplicate. Protein concentrations were measured by the Lowry method (24). Preparations of canine heart plasma membranes were assayed as a reference tissue. Values obtained for $-K_d$ and R_o using the heart membranes are in agreement with previous reports (18) (Table I).

Results. At 23° the association of [^{3}H]DHA binding to renal plasma membranes was rapid, reaching equilibrium within 5 min (Fig. 1). This equilibrium was maintained for at least 15 min. Following the addition of 1-propranolol to an equilibrated mixture of membrane and [^{3}H]DHA, dissociation of bound ligand was maximum in 3 min for outer cortical membranes and 5 min for inner cortical membranes.

The linearity of [^{3}H]DHA binding to renal plasma membranes at various protein concentrations is shown in Fig. 2. This figure reveals a difference in the binding capacities of the two cortical regions. At each protein concentration the inner cortex exhibited a greater ability to bind the radioligand than the outer cortex.

Specific binding of [^{3}H]DHA to partially purified renal plasma membranes was saturable for both the inner and the outer cortical areas. Scatchard plot analysis of the binding data showed two distinctly different linear

TABLE I. TOTAL RECEPTOR OCCUPANCY (R_o) AND DISSOCIATION CONSTANTS ($-K_d$) OF [^{3}H]DHA BINDING TO PARTIALLY PURIFIED PLASMA MEMBRANES FROM HEART AND FROM THE INNER AND OUTER CORTICAL REGIONS OF THE KIDNEY[a]

Tissue	*N*	R_o (pmole/mg protein)	$-K_d$ n*M*
Outer cortex	(4)	0.5216 ±0.0067	80.52 ±3.88
Inner cortex	(4)	0.4558 ±0.0319	37.33 ±1.55*
Heart	(7)	0.3573 ±0.0196	13.27 ±1.65

[a] The results are expressed as the mean ± SE. *N* represents the number of samples assayed. Statistical differences between means were assessed using the Student's *t* test (two-tailed test).

* $P < 0.001$ between the outer and the inner cortex.

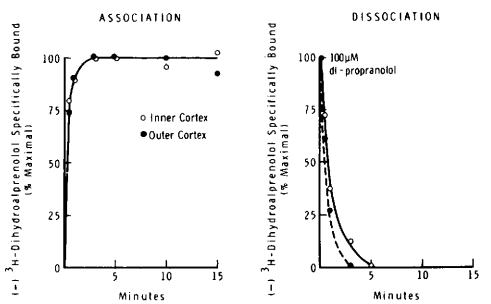

FIG. 1. Association and dissociation kinetics for inner and outer renal cortical membranes. Each point represents the mean of triplicate determinations from two separate assays.

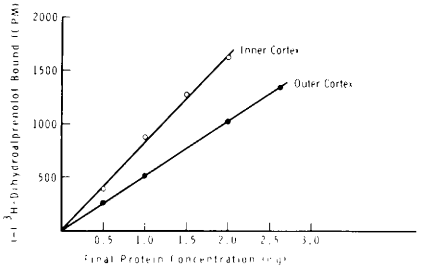

FIG. 2. Protein-dependent binding of [^{3}H]DHA to renal plasma membranes. Each point represents the mean of triplicate determinations from two separate assays. Linear regression analysis of the data points gives a line for the inner cortex as $y = 8.6 + 830x$ ($r = 0.9992$) and for the inner cortex as $y = 6.08 + 506x$ ($r = 0.9998$).

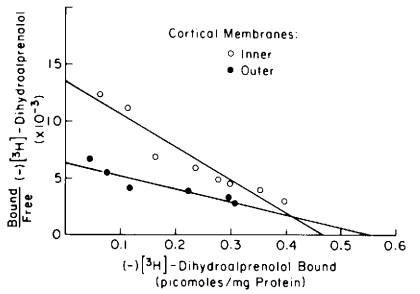

FIG. 3. Scatchard plot of (−)-[^{3}H]dihydroalprenolol binding to renal plasma membranes from the inner and the outer renal cortical areas. Analysis of the data points yields a linear regression line of $y = 0.0133 - 0.0281x$ for the inner cortex ($r = 0.9515$) and $y = 0.0064 - 0.0116x$ for the outer cortex ($r = 0.9243$).

regression lines (Fig. 3). A greater slope (or affinity) was seen for inner cortical than outer cortical membranes ($P < 0.05$). Table I shows the mean values for each cortical region. Although total receptor occupancy was slightly but not significantly greater in outer cortical membranes, the dissociation constant was approximately twofold higher in outer cortical membranes. The greater binding capacity demonstrated for inner cortical membranes therefore is due to the greater affinity of these membranes rather than the number of receptor sites.

Hill coefficients of the binding data for each renal plasma membrane approximated 1, indicating the absence of cooperative binding. Additional studies using this same procedure showed β-adrenergic stereospecificity for both membranes; l-propranolol was approximately 100-fold more effective in displacing [^{3}H]DHA than the D-isomer. Moreover, the α-adrenergic antagonist, phentolamine, was relatively ineffective in displacing the radioligand from either cortical membrane (Fig. 4).

Discussion. There is substantial evidence to suggest a role for β-adrenergic receptors in sodium reabsorption (via cAMP) at different nephron sites (3, 12, 25). Since the kidney has been shown to be heterogenous in terms of adrenergic innervation (16), renin concentrations (26), and nephron function (13), the presence of distinct differences in the binding of [^{3}H]DHA to inner and outer cortical membranes may represent an additional functional difference in regional activities of the kidney.

Investigations by Atlas *et al.* (27) using a fluorescent β-adrenergic antagonist, 9-amino-acridic propranolol, have shown β-adrenergic receptor binding sites in the epithelium of the proximal, distal, and collecting duct portions of the rat nephron as well as in the juxtaglomerular areas. However, these authors did not distinguish the intracortical location of these receptors.

Recently Eliahou *et al.* (28) using a similar membrane preparation technique have characterized [^{3}H]DHA binding sites in rat kidney tissues. Their studies showed a $-K_d$ of 9.3 nM and a R_o of 0.0695 pmole/mg of protein in plasma membranes prepared from whole kidneys. In their study, whole kidney homogenate rather than cortical tissue was assayed. Since binding sites appear primarily located in the proximal and distal tubules (27) preparations of cortical tissues should contain a greater concentration of β-adrenergic receptors than whole kidney. Differences between

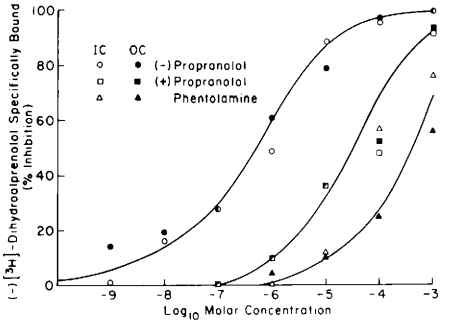

FIG. 4. Stereospecificity of [^{3}H]DHA binding to inner (open circles) and outer (closed symbols) cortical membranes. Each point represents the mean from three assays determined in triplicate.

our findings and theirs may also be a reflection of the incubation temperatures used (23 vs 37°), or species variations. Although a change in β-receptor response with temperature has been reported (decreases in temperature tend to reduce β- and increase α-receptors), this phenomenon occurs mainly at temperatures below 23° (29). Moreover, these effects appear related to changes in cAMP versus cGMP production rather than receptor populations (30).

Recent evidence has indicated that the kidney contains both β_1- and β_2-type subpopulations of β-adrenergic receptors (31). Burnett *et al.* (19) have reported that in the lung the ratio of β_1- to β_2-type receptors determines the affinity for binding; this raises the possibility that variations in tubular receptor affinities observed in different areas of the kidney cortex may be due to differences in the relative amounts of these β-adrenergic subtypes. It has been demonstrated in the brain (32) that the number of β-B_1 receptors is regulated by neurogenic activity whereas the β-B_2 receptors are apparently independent. If this relationship holds as well in the kidney determation of β-receptor subtypes may further define the role of neurogenic activity in sodium reabsorption.

Summary. β-Adrenergic receptors were characterized from plasma membranes obtained from two different regions of the canine kidney cortex by studying their binding of the radiolabeled β-adrenergic antagonist [^{3}H]dihydroalprenolol. Total receptor occupancy was slightly but not significantly greater in outer cortical membranes; the dissociation constant was approximately twofold higher in the outer cortical membranes. A greater binding capacity demonstrated for inner cortical membranes was due to greater affinity of the receptors in this area rather than a difference in receptor sites. β-Adrenergic stereospecificity was demonstrated in both membranes; l-propranolol was more effective in displacing [^{3}H]DHA than D-propranolol.

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