Distribution of $\alpha_1$- and $\alpha_2$-Adrenoceptors in Brush Border and Basolateral Membranes from Rat Kidney Cortical Tubules

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Abstract—To examine the localization of $\alpha$-adrenoceptors within the kidney tubule, binding experiments with $^{125}$I-HEAT and $^3$H-yohimbine were performed using cortical homogenates, basolateral membranes (BLM) and brush border membranes (BBM). $B_{\text{max}}$ values for $^{125}$I-HEAT or $^3$H-yohimbine binding were 2.3- or 2.0-fold that of the homogenate in BBM and 8.2- or 10.7-fold in BLM, respectively. $K_i$ values for $^{125}$I-HEAT or $^3$H-yohimbine were not significantly different between BBM and BLM. These results indicate that both types of $\alpha$-adrenoceptors seem to be localized largely in the BLM.

Morphological studies revealed that nerve terminals make direct contact with the basement membranes of rat kidney proximal tubules (1). $\alpha_1$- and $\alpha_2$-adrenoceptors in crude renal membranes have been identified by the radioligand binding technique (2-6). Autoradiographic studies demonstrated that the most of these receptors are largely confined to the proximal tubules of the kidney (7, 8), and functional studies (9, 10) and radioligand experiments (11) using microdissected proximal tubules revealed the existence of $\alpha$-adrenoceptors.

Kidney tubules consist of two types of distinct characteristic cell membranes with regard to function and structure; i.e., brush border membranes (BBM) and basolateral membranes (BLM). Thus, determination of the anatomical localization of the receptor would lead to a better understanding of adrenergic mechanisms in the kidney. The localization of $\alpha_1$- and $\alpha_2$-adrenoceptors within the proximal tubules has not been clearly defined because most of the radioligand binding studies were done using crude renal membranes.

We have recently shown with BLM, the existence and characterization of 2-(8-3-1$^{25}$I-4-hydroxyphenyl)-tetralone ($^{125}$I-HEAT, an $\alpha_1$-antagonist) and $^3$H-yohimbine (an $\alpha_2$-antagonist) binding sites which seemed to be $\alpha_1$- and $\alpha_2$-adrenoceptors (12). In the present study, $^{125}$I-HEAT and $^3$H-yohimbine binding to isolated BBM was compared with that to BLM in order to determine the distribution of these $\alpha$-adrenoceptors within the tubules. In addition, changes in receptor densities and also in marker enzyme activities were examined, among the initial homogenates, BLM and BBM.

BLM and BBM were simultaneously isolated from the kidney cortex of male Wistar rats (250-300 g), using a combination of Percoll (Pharmacia) density gradient centrifugation with calcium precipitation. Briefly, the kidney cortex was homogenized in 0.25 M sucrose containing 0.1 mM PMSF (phenylmethyl sulfonyl fluoride) and 10 mM Hepes (N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid)-Tris, pH 7.4 (sucrose buffer). To remove cell debris and unbroken cells, homogenates were spun at 50×g for 15 min. The supernatants thus obtained were tentatively considered initial homogenates, and aliquots of the supernatants were used to examine various marker enzyme activities and the $\alpha$-adrenoceptor. After Percoll density gradient centrifugation (total volume 30 ml), $(\text{Na}^+ + \text{K}^+)$-ATPase (BLM enzyme) activity was found in the upper part of
the gradient and the BBM marker enzyme γ-GTP (γ-glutamyltranspeptidase) migrated toward the bottom of the Percoll gradient, as previously reported (12). The upper 9–14 ml containing the fraction enriched in BLM were pooled and diluted 2-fold with sucrose buffer. The lower 21–30 ml containing the fraction enriched in BBM were collected and diluted 5-fold with 10 mM mannitol and 10 mM Hepes-Tris, pH 7.4. Both fractions of BLM and crude BBM were centrifuged at 105,000 × g for 60 min to remove the Percoll. BLM pellets were pooled and resuspended in sucrose buffer (12). After the crude BBM had been resuspended in 10 mM mannitol and 10 mM Hepes-Tris, 10 mM CaCl₂ was added and the preparation mixed at 0°C. The preparations were allowed to stand for 10 min and then centrifuged at 1,500 × g for 15 min to sediment the cell organelles and contaminant BLM. The resultant supernatants were re-centrifuged at 48,000 × g for 30 min. Membrane pellets thus obtained were re-suspended in the sucrose buffer. Both BLM and BBM were stored at -70°C until use in the binding experiments.

The marker enzyme activities in the initial homogenates, BLM and BBM were determined as described (12). Each marker enzyme activity was determined using fresh, not frozen samples. Protein was measured by the method of Bradford (13), using bovine serum albumin as standard.

The bindings of 125I-HEAT (NEN) or 3H-yohimbine (Amersham) to homogenates, BLM and BBM were generally performed as described (12). Protein concentrations in the incubation medium (total volume 150 μl) were ca. 4 (initial homogenates) or 1 (BBM) for yohimbine and 0.3 (initial homogenates) or 0.3 mg/ml (BBM) for HEAT. Both of the radioligands bound linearly within these protein concentrations in the respective membrane. Initial homogenates, BLM or BBM were incubated at 25°C for 30 min with the radioligands, as described (12). Specific binding was defined as the difference between total binding minus nonspecific binding. Nonspecific binding was determined as the amount of 125I-HEAT or 3H-yohimbine bound in the presence of 10 μM phentolamine (gift from Nihon Ciba-Geigy).

There were vesicular membranes and no apparent fragmented microvilli in the electron micrographs of isolated BLM and BBM fractions consisting of fragmented microvilli and vesicular membranes (data not shown). Specific activities and relative enrichment of various marker enzymes in the BLM and BBM, compared with those of initial homogenates, are shown in Table 1. As described (12), specific activity of (Na⁺+K⁺)-ATPase of the BLM was increased 10.2±0.8-fold over that of the initial homogenates, whereas other marker enzymes for mitochondria, cytoplasm, lysosome, and endoplasmic reticulum had low activities. (Na⁺+K⁺)-ATPase activity, a marker enzyme for BLM, of the BBM was slightly increased 1.3±0.4-fold over that in the initial homogenates. γ-GTP and AP-M activities (both are BBM enzymes) of this fraction were 11.7±1.0- or 19±0.8-fold over those of the initial homogenates. These results show that the BLM and BBM could

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<th>Table 1. Specific activities and relative enrichment of marker enzymes in BLM and BBM compared with initial homogenate</th>
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<td>(Na⁺+K⁺)-ATPase*</td>
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<td>Homogenate</td>
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<td>BLM</td>
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γ-GTP, γ-glutamyltranspeptidase; AP-M, aminopeptidase-M; G-6-Pase, glucose-6-phosphatase; NAG, N-acetyl-β-D-glucosaminidase; LDH, lactate dehydrogenase; SDH, succinic dehydrogenase. Shown are the mean±S.E.M. of four preparations. *nmol/min per mg protein, **decrease in absorbance/min per mg protein, ***I.U./mg protein.
Table 2. Characteristics of 125I-HEAT or 3H-yohimbine binding to homogenates, BBM and BLM from rat kidney cortex.

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<th>125I-HEAT (n=4)</th>
<th>3H-yohimbine (n=4)</th>
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<tbody>
<tr>
<td></td>
<td>$K_d$ (nM)</td>
<td>$B_{max}$ (fmol/mg)</td>
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<tr>
<td>Homogenate</td>
<td>0.062±0.009</td>
<td>50.1±4.9</td>
</tr>
<tr>
<td>BBM (Enrichment)</td>
<td>0.083±0.008*</td>
<td>116.5±8.8**</td>
</tr>
<tr>
<td>BLM (Enrichment)</td>
<td>0.088±0.009*</td>
<td>409.8±2.3**</td>
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*Not significant and **significantly different (Student’s t-test, P<0.001) between BBM and BLM.

be isolated using these procedures.

Table 2 summarizes the characteristics of 125I-HEAT and 3H-yohimbine binding to homogenates, BBM and BLM. Bindings of both ligands to BLM, BBM and homogenate were saturable with time and ligand concentrations and reversible. In the present experiment, we confirmed that there was a high density of both types of $\alpha$-adrenoceptors in the BLM. $B_{max}$ values for 125I-HEAT binding increased to 2.3±0.4-fold over that in the homogenate in BBM and 8.2±0.5-fold in BLM, respectively. Similarly, $B_{max}$ values for 3H-yohimbine binding increased to 2.0±0.3-fold over the homogenate in BBM and 10.7±0.6-fold in BLM, respectively (Table 2). Enrichment of (Na\(^++\)K\(^+\))-ATPase activity (1.3±0.3-fold) in the BBM was not significantly different from that of $B_{max}$ values for 125I-HEAT (2.3±0.4-fold, P>0.05) or 3H-yohimbine (2.0±0.3-fold, P>0.1) binding to this fraction, respectively. In case of the BLM, there were no significant differences between enrichment of $B_{max}$ values for 125I-HEAT (8.2±0.5-fold, P>0.05) or 3H-yohimbine (10.7±0.6-fold, P>0.5) and that of (Na\(^++\)K\(^+\))-ATPase activity (10.2±0.8-fold, P>0.1), respectively. Thus, increases in receptor densities in both membrane fractions, compared with initial homogenates, parallel those in (Na\(^++\)K\(^+\))-ATPase activities, in both membranes. Detection of $\alpha_1$- and $\alpha_2$-adrenoceptors in BBM fractions suggests the following possibilities: 1) the BBM fraction may be contaminated with membranes from glomeruli or cortical collecting tubules. Alpha-adrenoceptors are present in these two tissues (9, 14–18); 2) Small amounts of adrenoceptors may be localized in this fraction; and 3) the BBM fraction is probably contaminated with proximal BLM which contain large amounts of $\alpha_1$- and $\alpha_2$-adrenoceptors. Although there are both $\alpha_1$- and $\alpha_2$-adrenoceptors in the membranes from rat kidney glomeruli (15, 18), the possibility that the BLM fraction may be contaminated with these membranes is negligible because glomeruli make up less than 5% of the total cortical mass and densities of $\alpha$-adrenoceptors are only 1- or 2-fold higher than those in the initial materials (15). Furthermore, $\alpha_2$-adrenoceptors are present in the cortical collecting tubules (9, 16, 17), but $\alpha_1$-adrenoceptors have not been detected in this segment. However, in the BBM fraction, both types of $\alpha$-adrenoceptors were enriched over the initial homogenate, suggesting that this fraction was not contaminated with cortical collecting tubular membranes. Alpha$_1$- and $\alpha_2$-adrenoceptors seen in BBM may be due to the contamination of BLM because the degree of contamination of BLM (enrichment of (Na\(^++\)K\(^+\))-ATPase activity over the initial homogenates) was not significantly different from that of increases in receptor densities, compared with initial homogenates (Tables 1 and 2). Chan (10) found in microperfusion experiment that the addition of norepinephrine to the capillary perfusate increased fluid absorption, but perfusion of the lumen with this agent was without effect. Our present findings that most of the receptors are localized in the BLM support this observation. DiBona and Sawin (19) have shown that low frequency renal nerve stimulation produces antidiuresis and antinatriuresis without decreasing glomerular filtration rate and renal blood flow in rats. These antidiuretic and
antinatriuretic responses were inhibited by prazosin, an α₁-antagonist, but were unaffected by rauwolscine, an α₂-antagonist (19), thereby indicating that α₁- not α₂-adrenoceptors regulate the water and electrolyte transport in the tubules. In contrast, α₂-adrenoceptors in the proximal tubules were shown to attenuate the parathyroid hormone sensitive adenylate cyclase activity (9). Thus, we postulate that α-adrenoceptors of the BLM mediate the transport of water and electrolytes.

We conclude that α₁- and α₂-adrenoceptors are mainly localized in peritubular membranes. Whether or not BBM contain some α-adrenoceptors with the same affinity seen in the BLM remains the subject of further study.

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References