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CHARACTERIZATION OF α_1- AND α_2-ADRENOCEPTORS DIRECTLY ASSOCIATED WITH BASOLATERAL MEMBRANES FROM RAT KIDNEY PROXIMAL TUBULES

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Abstract—We have used 2-(β-(3-^{125}iodo-4-hydroxyphenyl)-ethylaminoethyl)-tetralone ([^{125}I]HEAT or BE2254), an α_1-selective antagonist, and [^{3}H]yohimbine, an α_2-selective antagonist, to demonstrate and characterize binding sites in basolateral membranes from rat kidney cortex. Parathyroid hormone (PTH) stimulated the adenylate cyclase activity of the basolateral membranes, whereas thyrocalcitonin, arginine vasopressin (AVP) and isoproterenol did not. Therefore, the basolateral membranes were probably derived from the proximal tubules. The specific binding of [^{125}I]HEAT and [^{3}H]yohimbine to basolateral membranes was rapid, reversible, saturable and of high affinity. The maximum densities of α_1- and α_2-receptors were 364 and 1130 fmoles/mg protein, indicating that the ratio of α_1- to α_2-adrenoceptors was about 1:3. The specific binding of [^{125}I]HEAT and [^{3}H]yohimbine to the basolateral membranes was displaced by various adrenergic agents in a manner that suggests that the labeled sites probably represent α_1- and α_2-adrenoceptors respectively. These results suggest that the binding sites of [^{125}I]HEAT and [^{3}H]yohimbine, which appear to be α_1- and α_2-adrenoceptors, exist in the basolateral membranes of the proximal tubules.

Renal nerves play an important role in the regulation of tubular functions [1–4]. Morphological studies demonstrate adrenergic nerve terminals in direct contact with basement membranes of the proximal and distal tubules in monkey [5], dog [2], and rat [6] kidney. α_1-, α_2-, β_1- and β_2-Adrenoceptors have been identified in the kidney, using radioligand-binding techniques [7–12]. However, the sites of action of α-adrenergic agents along the nephron have not been defined clearly. Localization of [^{3}H]prazosin (α_1-antagonist) and [^{3}H]rauwolscine or [^{3}H]clonidine (both α_2-selective agents) binding in the kidney, demonstrated by autoradiography, shows that most sites are largely associated with proximal tubules [13, 14]. On the other hand, it was reported that the Madin–Darby canine kidney (MDCK) cells which are derived from distal and collecting tubules possess α_1-adrenoceptors [12]. In addition, Chabardès *et al.* [15] and Krothapalli and Suki [16] have shown functional characterization of α_2-adrenoceptors in cortical collecting tubules of rat and rabbit kidney respectively. Moreover, Chan [17] reported that net water flux of the rat proximal convoluted tubules stimulated with norepinephrine is inhibited by phenoxybenzamine but not by propranolol, thereby suggesting the existence of α-adrenoceptors in the proximal convoluted tubules. In contrast, Bello-Reuss [18] stated that the catecholamine-induced increase in net water flux of proximal tubules is due to stimulation of β-adrenoceptors.

In addition to the contradictory reports concerning the adrenoceptors in the proximal tubules, details on biochemical characterization and identification of α_1- and α_2-adrenoceptors in the proximal tubular basolateral membranes of rat kidney are poorly understood because the data available are derived from binding studies using only crude renal membranes, which may be composed of the different types of tubular cell membranes (proximal, distal and cortical collecting tubules) and renal vascular cell membranes. Scalera *et al.* [19] developed the method for isolating basolateral membranes from rat kidney cortex. Basolateral membranes prepared by this method are exclusively derived from proximal tubules in the rat [19] and dog [20]. We now report the identification and biochemical characterization of the α_1- and α_2-adrenoceptors in the proximal tubular basolateral membranes.

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MATERIALS AND METHODS

Membrane preparation

Kidneys from male Wistar rats, weighing 250–350 g, were obtained immediately after decapitation and were then placed in an ice-cold sucrose buffer (0.25 M sucrose, 0.1 mM PMSF (phenylmethyl sulfonyl fluoride) and 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–Tris, pH 7.4). All subsequent preparative steps were performed on ice or at 4°. After removing the capsules and bisecting the kidneys longitudinally, cortical tissues were obtained. The combined tissues of two or three rats were weighed and rinsed with sucrose buffer. The tubular basolateral membranes were then isolated from these cortical tissues by differential centrifugation and Percoll density gradient centrifugation, using the methods of Scalera *et al.* [19] with slight modification. After obtaining the heavy microsomal fraction, Percoll was added to a

final concentration of 10% (v/v), and the suspension (final volume, 30 ml) was centrifuged at 48,000 *g* for 30 min. Initially, and periodically thereafter, density marker beads were added before centrifugation, and the position of the basolateral membranes in the gradient was determined by enzyme analyses of each 1 ml of gradient. When the preparations were used for binding studies, 10 μl of blue (1.037 g/ml) density marker beads was added to parallel control tubes (containing sucrose buffer and Percoll) to monitor the position of the membranes in the gradient. As reported by Scalera *et al.* [19] and confirmed in our laboratory, the brush border enzyme alkaline phosphatase (data not shown) and γ-GTP (γ-glutamyltranspeptidase) migrate toward the bottom of the Percoll gradient. $(Na^+ + K^+)$-ATPase activity was found in the upper part of the gradient. The upper 9–14 ml containing the fraction enriched in basolateral membranes was pooled, diluted 2-fold with sucrose buffer, and centrifuged at 105,000 *g* for 60 min to remove the Percoll and pellet the membranes. The loose membrane pellets were pooled, resuspended in the sucrose buffer, and stored at −70° until use for binding experiments.

Enzyme and protein determination

$(Na^+ + K^+)$-ATPase. Basolateral membrane purification was assessed from the enrichment of $(Na^+ + K^+)$-ATPase activity. In order to open closed vesicles, the technique of Jørgensen [21] was applied. Homogenate and membrane fractions suspended in the sucrose buffer and containing 0.5 to 1 mg per ml were treated with a mixture of deoxycholate and EDTA (ethylenediaminetetraacetic acid) (final concentrations 0.09% and 2 mM respectively) for 30 min at room temperature. Immediately after this treatment, the samples were tested for enzyme activity. The incubation medium, final volume of 1 ml, contained 100 mM NaCl, 20 mM KCl, 2 mM $MgCl_2$, Na_2-ATP (pH adjusted to 7.4), 10 mM Tris–HCl buffer (pH 7.4) and, where appropriate, 2 mM ouabain. The final quantity of protein was between 10 and 30 μg, in which range the ATPase activity revealed a linear relationship with the concentration. The incubation medium was prewarmed for 5 min at 37° and the reaction was started by addition of the enzymes. After 10 min, the reaction was stopped by the addition of 0.5 ml of 15% trichloroacetic acid. The samples were mixed and centrifuged, and the orthophosphate in the supernatant fraction was determined [22]. Preliminary experiments showed that under these conditions, there was a linear relationship between the quantity of phosphate liberated and the incubation time. All samples were run in duplicate. The ATPase activity is expressed as nmoles of phosphate liberated per mg protein per min, after subtraction of a blank in which the samples were added to the incubation medium only after trichloroacetic acid. The activity of $(Na^+ + K^+)$-ATPase was obtained by subtracting the activity found in the presence of ouabain from the total ATPase activity, measured in the absence of ouabain.

Other marker enzymes. Aminopeptidase M (AP-M) and γ-glutamyltranspeptidase (γ-GTP), used as markers of brush border membranes, were determined as described [23, 24] respectively. *N*-Acetyl-β-D-glucosaminidase (NAG), a marker of lysosome, glucose-6-phosphatase (G-6-Pase), a marker for endoplasmic reticulum, succinate dehydrogenase (SDH), a marker for mitochondria, and lactate dehydrogenase (LDH), a marker for cytosol, were assayed as described [25–28] respectively.

Measurement of adenylate cyclase activity. Adenylate cyclase activity was measured by the conversion of ATP to cAMP, according to Torikai *et al.* [29] with slight modification. Briefly, 20 μl of hypotonic preincubation medium [8 mM Tris–HCl (pH 7.4), 1 mM $MgCl_2$, 0.25 mM EDTA, and 0.1% bovine serum albumin) was added to 20 μl of basolateral membranes (15–20 μg protein). This preincubation medium contained appropriate test hormones or the vehicle at a concentration of 5 μM. After 30 min of preincubation of 0°, the 60 μl of incubation medium added consisted of 100 mM Tris–HCl (pH 7.4), 3.8 mM $MgCl_2$, 0.25 mM EDTA, 1.35 mM 1-methyl-3-isobutylxanthine, 0.6 mM ATP, 20 mM phosphocreatine, and 1 mg/ml creatine phosphokinase. The reaction was carried out at 37° for 30 min and was then terminated by the addition of 1 ml of 5% trichloroacetic acid; the mixture was vortexed and extracted three times with 5 ml of water-saturated ether. The residual aqueous phase was succinylated by anhydrous succinate, dioxane and triethylamine. The cAMP formed was measured by radioimmunoassay, using commercial kits. All of the enzyme activities tested were linear with time and membrane concentrations.

Protein was measured by the method of Benzadoun and Weinstein [30] using bovine serum albumin as standard.

Binding studies.

Binding studies were performed generally as described by Williams *et al.* [31] using [^{125}I]HEAT and [^{3}H]yohimbine. Basolateral membranes (protein concentration, 50 μg/ml for [^{125}I]HEAT binding or 1 mg/ml for yohimbine binding) were incubated with the radioligand at 25° for 30 min (unless otherwise indicated) in the buffer containing 167 mM sucrose and 6.7 mM HEPES–Tris (pH 7.4), and 50 mM Tris–HCl (pH 7.4) and 10 mM $MgCl_2$ for [^{125}I]HEAT or 50 mM Na_2HPO_4 and 50 mM KH_2PO_4 (pH 7.4) for [^{3}H]yohimbine, in a final volume of 150 μl. Incubations were run in duplicate, and the incubation was terminated by the addition of 1 ml of ice-cold stop solution containing 50 mM Tris–HCl and 10 mM $MgCl_2$ for [^{125}I]HEAT and 50 mM phosphate buffer for [^{3}H]yohimbine (pH 7.4). Each sample was quickly filtered under vacuum through Whatman GF/F filters. The filters were then washed with 15 ml of ice-cold stop solution. Bound radioactivity was determined by counting the filters in a Packard gamma counter or a Beckman scintillation spectrometer (model LS 3800). Counting efficiency of ^{125}I or ^{3}H was 70 or 47% respectively. Specific binding was defined as the difference between total binding minus nonspecific binding and represented 85–90% of total binding for both ligands. Nonspecific binding was determined as the amount of [^{125}I]HEAT or [^{3}H]yohimbine bound in the presence of 10 μM phentolamine.

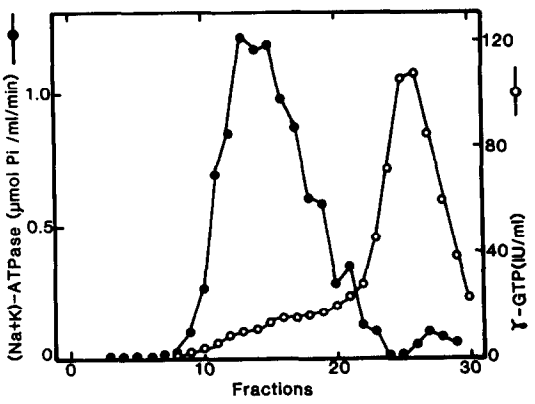

Fig. 1. Distribution of marker enzymes of basolateral membranes in the Percoll gradient.

Calculation

To determine the density and affinity of α_1- and α_2-adrenoceptors, Scatchard analyses were performed. Basolateral membranes were incubated with [^{125}I]HEAT (0.02 to 0.64 nM) or [^{3}H]yohimbine (1.5 to 33 nM) for 30 min at 25°.

The abilities of various adrenergic antagonists and agonists to compete for [^{125}I]HEAT or [^{3}H]yohimbine binding to basolateral membranes were also determined. The equilibrium dissociation constant, K_i, for the interaction of each compound with the binding site was calculated from the concentration of each agent which caused 50% maximum displacement (IC_{50}), using the equation of Cheng and Prusoff [32]:

$$K_i = IC_{50}/[1 + a/K_d]$$

where a is the concentrations of radioligands in the assay and K_d is the equilibrium dissociation constant for [^{125}I]HEAT or [^{3}H]yohimbine calculated from equilibrium binding studies mentioned above. Values for IC_{50} were estimated as described by Jarrott *et al.* [33].

Drugs

Norepinephrine hydrochloride, isoproterenol hemisulfate, phenylephrine hydrochloride, propranolol hydrochloride, yohimbine hydrochloride, dopamine hydrochloride, clonidine hydrochloride, arginine vasopressin, salmon calcitonin, parathyroid hormone (1–34 bovine synthetic) and PMSF were purchased from Sigma. Phentolamine mesylate and prazosin hydrochloride were provided by Ciba-Geigy (Takarazuka, Japan) and Pfizer Taito (Japan) respectively. Percoll and density marker beads were obtained from Pharmacia Fine Chemicals. Other chemicals used were of reagent grade. [O-*methyl*-^{3}H]Yohombine (72 Ci/mmole) and [^{125}I]HEAT (BE2254), (2088 Ci/mmole) were obtained from Amersham (Buckinghamshire, England). Cyclic AMP assay kits were obtained from Yamasa Shoyu (Japan).

Table 2. Effects of PTH, AVP, calcitonin and isoproterenol on adenylate cyclase activity of basolateral membranes

Additions	cAMP formed (pmoles/mg protein/30 min)
Control	321 ± 86
PTH	1248 ± 198
AVP	352 ± 48
Isoproterenol	362 ± 81
Calcitonin	421 ± 88

Shown are the mean ± S.E. of three different membrane preparations. Further details are in the text.

RESULTS

Isolation and characteristics of basolateral membranes

Figure 1 shows the distribution of $(Na^+ + K^+)$-ATPase, a marker enzyme for basolateral membranes, and γ-GTP, a marker for brush border membranes, after centrifugation in the Percoll medium. Maximal $(Na^+ + K^+)$-ATPase activity was found in the 13–15 ml from the top of the gradients at an approximate density of 1.037 g/ml, as determined with the use of density marker beads. In contrast, the peak of γ-GTP activity was 26–27 ml from the top of the gradient. The specific activities and relative enrichment of the various marker enzymes in the basolateral membranes, compared with those of initial homogenates, are shown in Table 1. Specific activity of $(Na^+ + K^+)$-ATPase of basolateral membranes was increased 10.2-fold over that of the homogenates. γ-GTP and AP-M activities of this fraction were very low. Other marker enzymes were also found in very low activities. Table 2 shows the effects of PTH, AVP, calcitonin and isoproterenol on adenylate cyclase activity of basolateral membrane fraction. PTH, but not the other hormones,

Table 1. Specific activities and relative enrichment of marker enzymes in basolateral membranes compared with initial homogenate

	$(Na^+ + K^+)$ATPase*	γ-GTP†	AP-M*	G-6-Pase*	NAG*	LDH‡	SDH‡
Homogenate	292 ± 29	1.85 ± 0.13	122 ± 4	94 ± 4	29.67 ± 1.96	0.797 ± 0.039	0.037 ± 0.003
Basolateral	2976 ± 154	2.66 ± 0.09	134 ± 9	196 ± 16	14.24 ± 1.08	0.225 ± 0.009	0.012 ± 0.001
Enrichment	10.2	1.4	1.1	2.1	0.5	0.3	0.3

Shown are the mean ± S.E.M. of four preparations.
* Expressed as nmoles/min per mg protein.
† I.U./mg protein.
‡ Decrease in absorbance/min per mg protein.

enhanced the activity of adenylate cyclase. These results indicate that the fraction was enriched in basolateral membranes derived from the proximal tubules [34]. The protein recovered from basolateral membranes was 0.8%.

Binding kinetics

Binding of [^{125}I]HEAT and [^{3}H]yohimbine to basolateral membranes, as a function of time, is illustrated in Figs. 2 and 3. Binding of both ligands was saturable with time, reached an equilibrium within 15 min, and was rapidly reversible. In the case of [^{125}I]HEAT binding, a plot of $\ln[B_{eq}/(B_{eq} - B_t)]$ (where B_{eq} = binding at equilibrium and B_t = binding at time, t) versus time for association was linear and yielded an apparent association rate, or k_{obs}, of 0.3202 min^{-1} (Fig. 2B). The plot of $\ln[B_t/B_{eq}]$ versus time for dissociation likewise produced a straight line, indicating the expected first-order kinetics, with a slope equal to 0.1016 min^{-1}. This corresponds to the dissociation rate constant of binding, or k_2 (Fig. 2C). From these two values, the association rate constant (k_1) and the kinetically derived equilibrium dissociation constant (K_d) were calculated using the following equations:

$$k_1 = (k_{obs} - k_2)/[\text{ligand concentration}]$$
$$= (0.3202\text{–}0.1016)/(0.106 \times 10^{-9})$$
$$= 2.061 \times 10^9 \text{ M min}^{-1}$$
$$K_d = k_2/k_1$$
$$= 0.1016/(2.061 \times 10^9)$$
$$= 0.049 \text{ nM}$$

In the case of [^{3}H]yohimbine binding, the association and dissociation rates were also determined, as shown in Fig. 3B and 3C.

$$k_1 = (0.6207\text{–}0.4612)/(2.76 \times 10^{-9})$$
$$= 0.0578 \times 10^9 \text{ M min}^{-1}$$
$$K_d = 0.4612/(0.0578 \times 10^9)$$
$$= 7.98 \text{ nM}$$

Association and dissociation of [^{3}H]yohimbine binding were more rapid than those of [^{125}I]HEAT binding.

Number and affinity of binding sites

To determine the number and affinity of [^{125}I]HEAT and [^{3}H]yohimbine binding sites of basolateral membranes, membranes were incubated with [^{125}I]HEAT or [^{3}H]yohimbine, in the absence and presence of 10 μM phentolamine (Figs. 4 and 5). Bindings of both radioligands to the basolateral membranes were saturable with ligand concentrations. To determine the binding maximum and affinity of each ligand, these data were replotted by Scatchard analyses (Figs. 4 and 5). B_{max} values for [^{125}I]HEAT and [^{3}H]yohimbine were 364 and 1130 fmoles/mg protein respectively. Thus, the ratio of density of α_1- to α_2-adrenoceptors was about 1:3. The dissociation constants (K_d) of [^{125}I]HEAT and [^{3}H]yohimbine binding to basolateral membranes were 0.069 and 8.8 nM respectively. These values are in a good agreement with the results obtained from the binding kinetics.

Specificity of binding

Adrenergic agonists and antagonists competed for the [^{125}I]HEAT binding sites of basolateral membranes in relative affinities: norepinephrine (K_i = 518 nM) ≥ clonidine (610 nM) ≫ phenylephrine

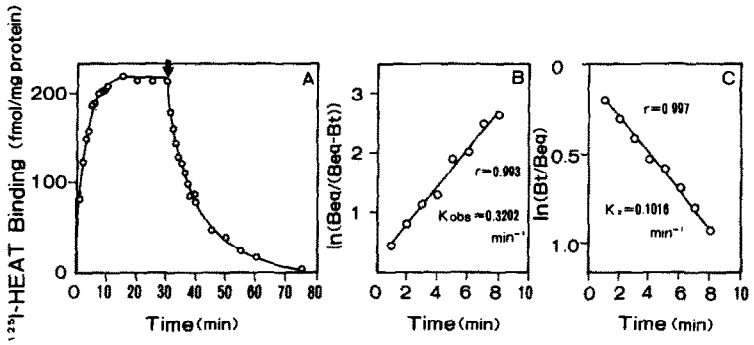

Fig. 2. Kinetics of [^{125}I]HEAT binding to basolateral membranes. (A) Membranes were prewarmed for 5 min at 25° and incubated batchwise with 0.106 nM [^{125}I]HEAT in two tubes in the absence (total binding) or the presence (nonspecific binding) of 10 μM phentolamine. At various times, duplicate 0.15-ml samples were removed from each tube and placed in smaller tubes containing 1 ml of cold stop solution. The samples were filtered and counted as described under Materials and Methods. For reversal of binding, a final concentration of 10 μM phentolamine was added to the total binding tube at 30 min (arrow). Sampling was carried out as mentioned above. This figure is representative of three experiments. (B) Analysis of kinetics of association of [^{125}I]HEAT binding with basolateral membranes. Specific binding data from A are plotted as $\ln[B_{eq}/(B_{eq}-B_t)]$ versus time, where B_{eq} = bound at equilibrium and B_t = bound at time t. (C) Analysis of kinetics of dissociation of [^{125}I]HEAT binding with basolateral membranes. Dissociation data from A are plotted as $\ln[B_t/B_{eq}]$ versus time. Definition of B_t and B_{eq} are the same as in B.

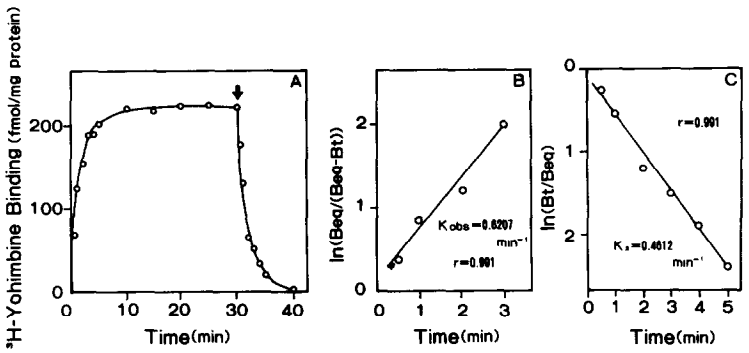

Fig. 3. Kinetics of [^{3}H]yohimbine binding to basolateral membranes. (A) This experiment was carried out the same as described for Fig. 2, except that [^{3}H]yohimbine (2.76 nM) was used instead of [^{125}I]HEAT. (B) Analysis of kinetics of association of [^{3}H]yohimbine with basolateral membranes, as described for Fig. 2B. (C) Analysis of kinetics of dissociation of [^{3}H]yohimbine with basolateral membranes, as described for Fig. 2C.

(3,725 nM) ≫ isoproterenol (87,960 nM) > dopamine (112,784 nM) (agonist) and prazosin (0.36 nM) ≫ phentolamine (8.99 nM) ≫ yohimbine (537 nM) ≫ propranolol (37,317 nM) (antagonist) (Fig. 6). The specificity of [^{3}H]yohimbine binding to basolateral membranes was also examined. The relative affinities for inhibition of [^{3}H]yohimbine binding by adrenergic agonists were as follows: clonidine (66 nM) ≫ norepinephrine (1,390 nM) ≫ phenylephrine (15,600 nM) ≫ dopamine (20,900 nM) > isoproterenol (109,300 nM) (Fig. 7A). Moreover, yohimbine (K_i = 7.5 nM) ≫ phentolamine (45 nM) > prazosin (72 nM) ≫ propranolol (8,764 nM) competed for the [^{3}H]yohimbine binding sites, in this order (Fig. 7B). These orders of relative affinities are identical with the well-known ones in eliciting physiological α_1- and α_2-adrenergic responses.

Distinction of binding and uptake

In the presence of osmotically sensitive membrane vesicles, membrane-associated [^{125}I]HEAT or [^{3}H]yohimbine may represent binding or transport into intravesicular spaces. To distinguish binding and transport, experiments with basolateral membranes were performed in which the osmotic pressure of the incubation medium varied by the addition of the impermeant solute sucrose. Figure 8 shows the independence of membrane-associated [^{125}I]HEAT and [^{3}H]yohimbine at a steady state from medium osmolarity. Since binding would be expected to be independent of osmotically sensitive intravesicular spaces, the observed relationship between medium osmolarity and membrane-associated [^{125}I]HEAT or [^{3}H]yohimbine demonstrates that 99% of the membrane-associated [^{125}I]HEAT or [^{3}H]yohimbine represents binding to the basolateral membranes and not translocation into vesicular spaces.

DISCUSSION

There is evidence for the existence and characterization of α-adrenoceptors in the renal crude membranes prepared from rat [9, 35–38] and guinea pig [33, 39]. However, these crude renal membranes

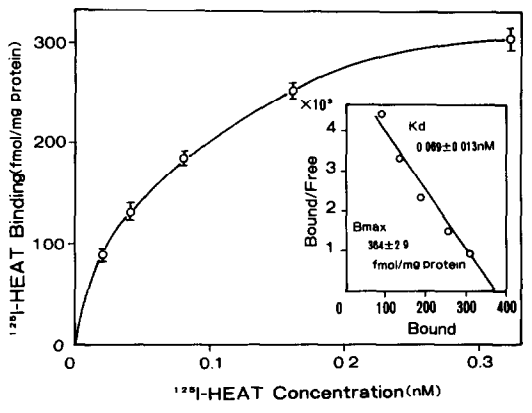

Fig. 4. Saturation binding isotherms for [^{125}I]HEAT on basolateral membranes. Membranes were incubated with various concentrations of [^{125}I]HEAT at 25° for 30 min in the absence (total binding) or presence (nonspecific binding) of 10 μM phentolamine. Each point represents the mean ± S.E. of three experiments.

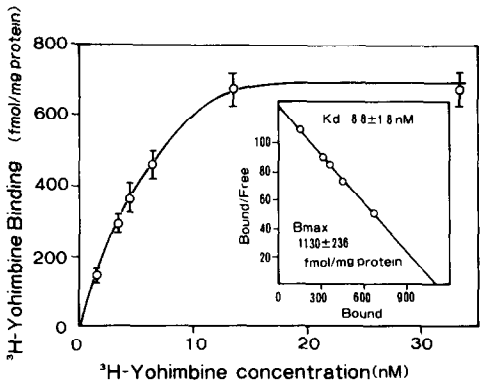

Fig. 5. Saturation binding isotherms for [^{3}H]yohimbine on basolateral membranes. This experiment was the same as that described for Fig. 4, except that [^{3}H]yohimbine was used instead of [^{125}I]HEAT. Each point represents the mean ± S.E. of four experiments.

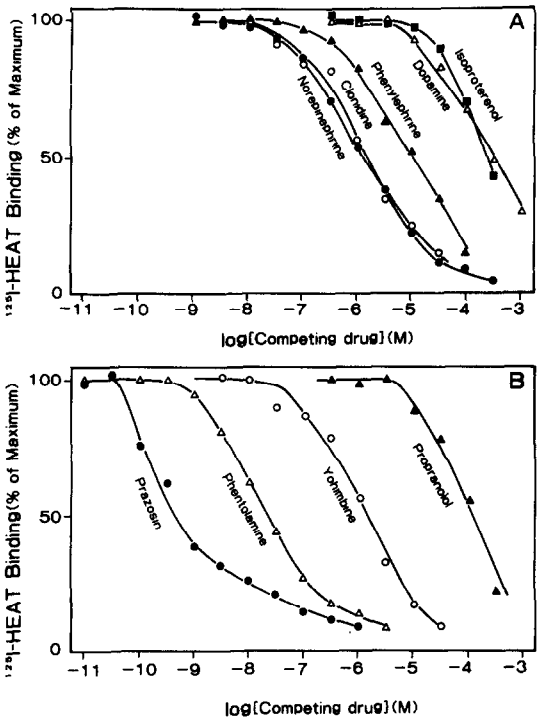

Fig. 6. Competition for [^{125}I]HEAT binding sites by adrenergic agonists (A) and antagonists (B). The binding of [^{125}I]HEAT (0.11 nM) to basolateral membranes was measured in the presence of the indicated concentrations of competing ligands. Data are expressed as a percentage of the maximum specific binding (186 fmoles/mg protein), as defined in the text. Each point represents the mean of two different preparations.

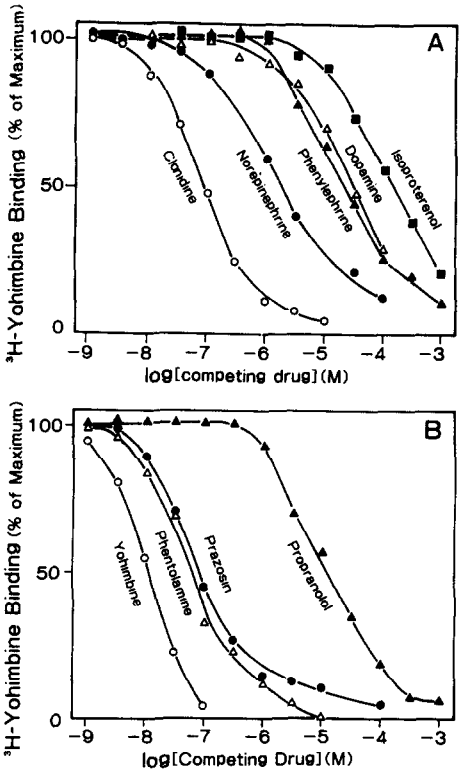

Fig. 7. Competition for [^{3}H]yohimbine binding sites by adrenergic agonists (A) and antagonists (B). These experiments were performed as described for Fig. 6, except that [^{3}H]yohimbine (3.0 nM) was used instead of [^{125}I]HEAT. Data are expressed as a percentage of maximum specific binding (285 fmoles/mg protein) as defined in the text. Each point represents the mean of two different preparations.

may contain renal vascular cell membranes and different types of tubular cell membranes. Although autoradiographic studies revealed that most binding sites of [^{3}H]prazosin, [^{3}H]rauwolscine or [^{3}H]clonidine are restricted to the proximal tubules of rat and guinea pig [13, 14], the stimulation of α_2-adrenoceptors by norepinephrine inhibited the vasopressin-induced adenylate cyclase activity in the cortical collecting tubules of rat and rabbit, thereby indicating the localization of α_2-receptors in this nephron segment [15, 16]. In addition, MDCK cells derived from distal tubules and collecting ducts have been shown to express α_1-adrenoceptors [12]. Thus, exact biochemical characterization and identification of α-adrenoceptors in the proximal tubules cannot be defined so long as crude renal membranes are used. Anatomical localization of the receptors would extend our understanding of adrenergic mechanisms in the kidney. Recently, Kusano *et al.* [40] demonstrated and partially characterized [^{3}H]prazosin binding sites in the isolated proximal convoluted tubule of rabbit kidney. On the other hand, Scalera *et al.* [19] and Schwab *et al.* [20] demonstrated that the basolateral membranes prepared from rat and dog kidney by Percoll density gradient centrifugation are derived from the proximal tubules. We also observed the stimulation of adenylate cyclase activity by only PTH and not by other hormones, as shown in Table 2, thus indicating that the basolateral membranes were derived from the proximal tubules [34]. Therefore, it is possible to determine which type of receptor is located in the proximal tubules and to

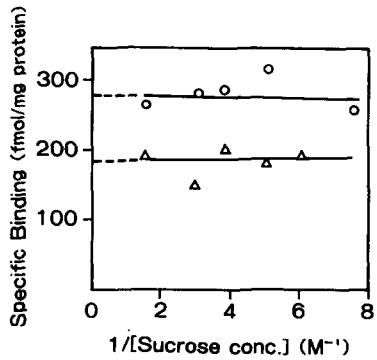

Fig. 8. Effect of medium osmolarity on basolateral membrane-associated [^{3}H]yohimbine. Basolateral membranes were prepared in 50 mM Tris–HCl buffer (for [^{125}I]HEAT binding) or 50 mM phosphate buffer (for [^{3}H]yohimbine binding). Binding was determined as a function of extravesicular sucrose concentration (133–667 mM). Circles and triangles represent the binding of [^{3}H]yohimbine and [^{125}I]HEAT respectively. The abscissa is the inverse of sucrose concentration (M) in the medium

study the characteristics of the receptors. The data presented in this paper suggest the presence of the α_1- and α_2-adrenoceptors with high affinity in the basolateral membranes of rat kidney proximal tubules.

The binding of [^{125}I]HEAT or [^{3}H]yohimbine to basolateral membranes was rapid and rapidly reversible (Figs. 2 and 3). However, both association and dissociation for [^{3}H]yohimbine binding were faster than those for [^{125}I]HEAT. These data agree with results which show that the binding of [^{3}H]prazosin is faster than that of [^{3}H]yohimbine [10]. The [^{125}I]HEAT and [^{3}H]yohimbine binding sites displayed high affinity (K_d, 0.069 nM for [^{125}I]HEAT and 8.8 nM for [^{3}H]yohimbine) (Figs. 4 and 5). These data are comparable with values reported by other investigators [9, 10, 33, 36, 38, 39, 41]. However, the binding site concentrations presented in this study were much higher than those reported by the investigators mentioned above (Figs. 4 and 5). This is probably due to use of the highly purified basolateral membranes as the starting materials. Schmitz *et al.* [9] and Snavely and Insel [10] have shown that the ratio of α_1- to α_2-adrenoceptor density is 1:3, using crude renal membranes. The present experiment demonstrated the ratio of α_1- to α_2-adrenoceptors to be 1:3 (Figs. 4 and 5). These results may be due to the fact that the most of the α-adrenoceptors are located in the proximal tubules [13, 14]. Therefore, much the same results were obtained with crude renal membranes or purified basolateral membranes.

The K_i value for prazosin (72 nM) in competition for yohimbine binding seems to be higher than that previously reported [42]. However, Snavely and Insel [10] have shown that the K_i value for prazosin in competition for yohimbine binding is 80 nM in rat kidney cortex, and our data are in agreement with these findings.

We also examined [^{125}I]HEAT and [^{3}H]yohimbine binding to the brush border membranes which were isolated by the calcium precipitation method from the bottom fraction of Percoll density gradient centrifugation (data not shown). The densities of [^{125}I]HEAT or [^{3}H]yohimbine binding to brush border membranes were less than those to the basolateral membranes. McPherson and Summers [43] have shown that [^{3}H]clonidine binding sites are concentrated in a fraction rich in basolateral membranes but not concentrated in brush border membranes of the guinea pig kidney. Furthermore, Chan [17] found that, in microperfusion experiments, the addition of norepinephrine to the capillary perfusate increased the fluid absorption, yet perfusion of the lumen with this agent was without effect. These observations seem to support our present data.

Our findings that the α_1- and α_2-receptors are distributed in basolateral membranes from proximal tubules are in good agreement with the results obtained by Chan [17]. Kusano *et al.* [40] also reported the presence of α-receptors in the proximal convoluted tubules. In the isolated perfused kidney, Smyth *et al.* [44] demonstrated that activation of α_2-adrenergic receptors with epinephrine decreases urinary cyclic AMP and sodium excretion without alteration in the perfusion pressure and glomerular filtration rate. Therefore, it is reasonable to assume that the α-receptors located on the proximal tubular membranes are associated with the metabolism of electrolytes and water. Accordingly, α_2-receptors in the proximal tubules seem to be associated with sodium transport. Further experiments are underway to clarify the mechanisms of direct control by the renal sympathetic nerves.

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