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BETA-ADRENERGIC RECEPTOR COUPLED ADENYLATE CYCLASE IN RAT KIDNEY

DIFFERENTIAL COUPLING IN GLOMERULI AND TUBULES

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Abstract—[^{125}I]Iodocyanopindolol ([^{125}I]ICYP) was used to identify and characterize the β-adrenoceptors in isolated rat kidney glomeruli and cortical tubules. In both the tissues, specific binding of [^{125}I]ICYP was saturable with time and ligand concentration and showed appropriate stereospecificity and agonist rank order potency characteristic of binding to β-adrenoceptors. Scatchard analysis revealed that the β-adrenoceptor concentration in the glomeruli (111.1 ± 8.9 fmol/mg protein) was about three times that in the tubules (40.1 ± 1.8 fmol/mg protein). The dissociation constants (K_D) were similar in the two tissues. Both β_1- and β_2-adrenoceptor subtypes were present in the glomeruli and tubules, but the β_1-subtype was predominant, constituting greater than 80% of the total β-adrenoceptors in the two tissues. Isoproterenol was twice as potent in competing for [^{125}I]ICYP binding in the tubules as in the glomeruli ($P < 0.05$). The slope factor (pseudo-Hill coefficient) for the isoproterenol competition curve was 0.74 ± 0.04 in the glomeruli and 0.54 ± 0.02 in the tubules ($P < 0.05$). The nonmetabolized guanyl nucleotide analogue Gpp(NH)p caused a steepening and a 3-fold shift of the isoproterenol competition curve in both tissues. Isoproterenol-stimulated cAMP accumulation in the glomeruli was only 31% of the value in the tubules. The concentration of isoproterenol producing half-maximal stimulation (EC_{50}) was 114 ± 13 nM in the glomeruli and 19 ± 3 nM in the tubules ($P < 0.05$). Gpp(NH)p and forskolin caused a similar increase in cAMP accumulation over basal value in the glomeruli as in the tubules. Overall, our results indicate a decreased efficiency in the interaction between the β-adrenergic agonist hormone, the receptor and the guanine nucleotide regulatory protein in the glomeruli as compared to the tubules.

There has been a growing appreciation, in the last decade, of the role of the β-adrenergic system in modulating many kidney functions. Electrical stimulation of sympathetic nerves leads to a β-adrenoceptor-mediated increase in renin secretion [1, 2]. β-Adrenergic vasodilation of renal vessels has been shown [1, 2]. The adrenergic system exerts important effects on renal tubular function. Denervation of the kidney leads to diuresis and natriuresis as well as to decreased reabsorption of glucose, phosphate, urate and other substances [1–3]. The exact nature of the adrenergic receptor mediating tubular reabsorption is controversial. In the isolated perfused rat kidney and proximal tubular segments, addition of catecholamines enhances sodium reabsorption via β-adrenergic receptors [4, 5]. In other preparations, evidence seems to suggest an α-receptor-mediated sodium reabsorption [2]. A nephron microdissection technique has demonstrated β-adrenoceptor-stimulated adenylate cyclase activity in the rat renal tubules, especially the distal tubules, cortical collecting tubules, and the thick ascending limb of the loop of Henle [3].

In recent years, direct binding studies with high-affinity radiolabeled ligands have been used to gain insight into the regulation and functioning of β-adrenergic receptors in many tissues [6–8]. Radioligand binding studies in the kidney reported in the literature have mostly used whole kidney cortex preparations [9, 10]. The kidney cortical preparation is a highly heterogeneous mixture of membranes from different cell types. Thus, while kidney cortical preparations are convenient to use, they do not provide any indication of whether the observations made are relevant to glomeruli, tubules, or both. Only two radioligand studies have been reported in which β-adrenergic receptors have been identified in isolated kidney preparations, one in tubules [11] and the other in glomeruli [12]. Both studies used [^{3}H]dihydroalprenolol to label the β-adrenergic receptors. The receptor number was not correlated with any functional activity in either of these studies.

In the present study, we report on the use of the iodinated β-adrenergic receptor antagonist ligand [^{125}I]iodocyanopindolol ([^{125}I]ICYP) to identify and characterize the β-adrenergic receptors in isolated rat kidney glomeruli and tubules. Our preliminary studies indicated that there may be important differences between the glomeruli and tubules with respect to the coupling between the β-adrenergic receptor and adenylate cyclase. This aspect has been investigated in some depth.

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MATERIALS AND METHODS

Materials. (−)[^{125}I]ICYP (sp. act. ~2200 Ci/mmol) was obtained from the New England Nuclear Corp. (Boston, MA). A [^{3}H]cAMP kit was obtained from the Diagnostic Products Corp. (Los Angeles, CA). (−)Alprenolol, (−)- and (+)isoproterenol bitartrate, (−)epinephrine bitartrate, (−)norepinephrine bitartrate, parathyroid hormone (PTH), arginine vasopressin (AVP), guanylyl imidodiphosphate [Gpp(NH)p] and forskolin were obtained from the Sigma Chemical Co. (St. Louis, MO). Betaxolol was a gift from Synthelabs (L.E.R.S.), Paris, France. All other chemicals were obtained from Sigma or from the VWR Scientific Co. and were analytical grade.

An E-C cellector tissue grinder (E-C cellector, 130 ml capacity, and fitting disposable 80 mesh screens for the cellector) was obtained from Thomas Scientific (Swedesboro, NJ). Stainless steel sieves, full height 5-inch diameter with pore sizes 212 μm, 125 μm and 63 μm, as well as a 5-inch diameter stainless steel receiver, were obtained from the Newark Wire Cloth Co. (Newark, NJ).

Experimental animals. Adult male Sprague–Dawley rats (Charles River, CD strain) weighing 200–250 g were used in all experiments. They were maintained on standard Purina Chow (Ralston Purina Co., St. Louis, MO) and were given free access to water. Experiments were done on unfasted animals.

Isolation of glomeruli and tubules. The isolation procedure was based on the techniques described by Price and Spiro [13] and Shah *et al.* [14] with minor modifications mainly in the sieving procedure that saved time and improved the purity of the obtained preparations. This procedure has the advantage of providing preparations of glomeruli and tubules from the same original cortical tissue. Briefly, the technique was: Following induction of anesthesia with ether, the kidneys were perfused with ice-cold isotonic (0.9%) saline at 7 ml/min until they were blanched. They were then removed and placed on an ice-cooled dissecting plate. All further preparative steps were performed at 4°. The renal capsule from each kidney was incised and removed from the kidney tissue. Each kidney was halved with a razor blade, and the medulla was removed. Cortical tissues from the kidneys of two to three rats were pooled for each experiment.

Glomeruli and tubule samples were prepared by a combination of sieving and differential centrifugation. The cortices were finely minced with a razor blade on the dissecting plate to the consistency of paste. This cortical paste was passed very gently through a 180 μm (80 mesh) stainless steel sieve (E-C Cellector Tissue Grinder) with a spatula, and the mash was collected in a plastic beaker containing 20 ml of isotonic saline placed under the sieve. The bottom of the sieve was then scraped with a clean spatula to recover as much of the mash as possible, and the volume of isotonic saline in the plastic beaker was brought up to 40 ml. The mash was then thoroughly suspended in the isotonic saline by forced ejection through a 22-gauge hypodermic needle. The resulting cortical suspension was collected in two 50-ml polycarbonate tubes. The volume in each tube was brought to 30 ml by the addition of ice-cold isotonic saline. The suspension of cortical tissue was then washed three times in isotonic saline by repeated centrifugation at 200 *g* for 3 min to eliminate the debris and small fragments floating in the supernatant fraction. The resulting pellet was resuspended in about 150 ml of isotonic saline in a plastic beaker.

Subsequent separation into glomeruli and tubules was achieved by the use of stainless steel sieves (Newark). These could be nested over each other making it possible to use large wash volumes and obtain purer preparations in a relatively short time. Before use, the sieves were cooled at 4° in the refrigerator and wetted with isotonic saline. For the sieving, the pellet in 150 ml isotonic saline was poured through the sieves nested over each other (212 μm, 125 μm and 63 μm top to bottom) with the receiver at the bottom. The nested sieves were gently shaken with the hands during the sieving procedure. The tubules collected in the 212 μm sieve while the glomeruli were retained by the 63 μm sieve. Subsequently, the 212 μm top sieve was rinsed with an additional 150 ml isotonic saline to remove any trapped glomeruli and smaller tissue fragments. The sieve was then removed and the retained tubules were recovered by inverting the screen over a plastic beaker and washing them out gently with 50 ml isotonic saline using a syringe. The 125 μm sieve was then rinsed with 150 ml isotonic saline to wash down any trapped glomeruli. The retained material in the 125 μm sieve consisted of small tubules and tissue fragments, and this was discarded. Finally, the glomeruli in the 63 μm sieve was further rinsed with 150 ml isotonic saline to wash down small tubular fragments. The retained glomeruli were then collected from the sieve in 50 ml isotonic saline in a similar manner as described for tubules above. The whole sieving procedure took less than 10 min. Glomerular and tubular preparations were then washed three times each at 150 *g* for 3 min, suspended in 5 ml isotonic saline, and checked for purity.

The purity of each glomerular suspension was evaluated by light microscopy and counting of glomeruli. An aliquot of glomerular suspension (20 μl) was stained on a microscope slide with 0.1% toluidine blue solution in isotonic saline to visualize cellular elements. It was examined under higher magnification (×400) to evaluate the structure of isolated glomeruli and then at lower magnification (×50) to determine the glomerular count and purity. Isolated glomeruli prepared by this procedure were generally without capsules, and the structure was fairly well preserved (Fig. 1). Occasionally small cell clusters, as well as a few ruptured glomeruli, were observed. The glomerular preparations routinely consisted of more than 97% glomeruli. The rest were small tissue fragments that could not be definitely identified. Tubules of the type seen in tubular fraction were not seen in the glomerular suspension. The tubular layer consisted of clumps of tubules. Glomeruli were rare (fewer than 2%).

In a few preliminary experiments, Krebs buffer was used in the isolation procedure instead of 0.9% saline. There was no difference in the morphological appearance of the glomeruli or the tubules with the

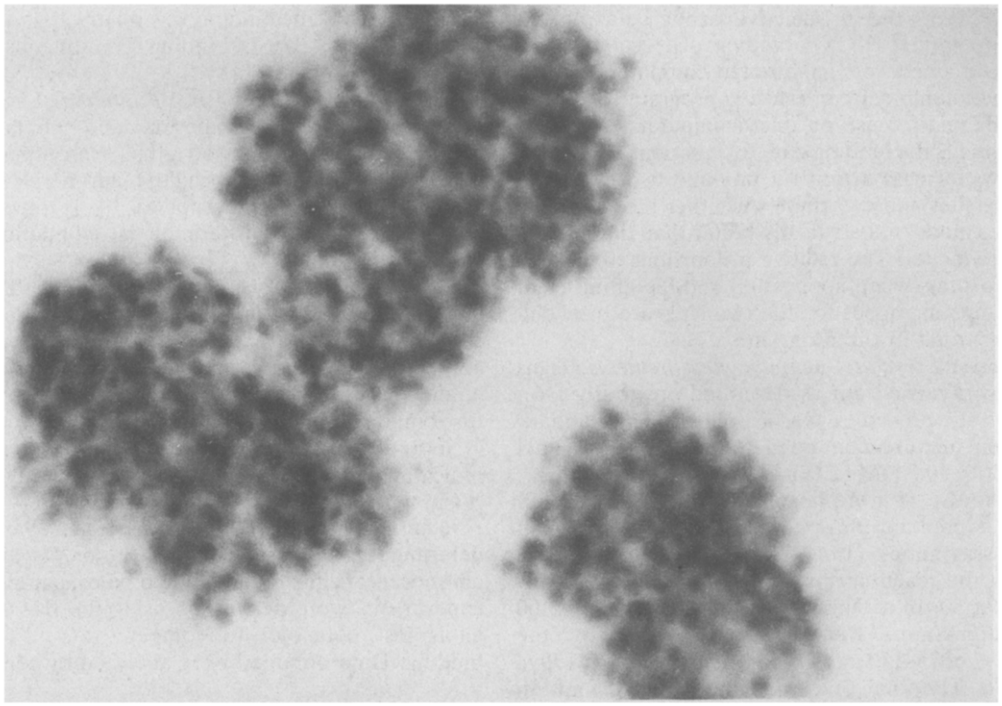

Fig. 1. Representative photomicrograph of isolated rat glomeruli. Glomeruli were obtained by sieving and differential centrifugation as described in Materials and Methods. Magnification ×400.

use of Krebs buffer, and the B_{max} and K_D values for [^{125}I]ICYP obtained in these glomeruli and tubules were similar to those obtained in the glomeruli and tubules isolated using 0.9% saline. The use of 0.9% saline was more convenient, and thus we used it for all the experiments reported in the current work. The use of isotonic saline for the glomerular isolation procedure has been reported earlier [13].

Preparation of glomerular and tubular particulate fractions. The technique was similar to the method used for preparing heart particulate fractions in our laboratory [15]. Isolated glomeruli and tubules suspended in isotonic saline were pelleted by centrifugation at ×2000 *g* for 5 min. The supernatant fluid was poured off, and the pellets were resuspended in 10 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4 at 25°) containing 1 mM ethyleneglycol-bis(amino-ethylether)tetra-acetate (EGTA). The tissues were then homogenized in a Brinkmann Polytron PT-20 homogenizer with three 8-sec bursts with the rheostat position at 5. The homogenate was then centrifuged for 15 min at ×48,000 *g*. The supernatant fraction was discarded, and the pellet was suspended in 10 ml of the same buffer and used fresh for the binding and adenylate cyclase assays.

Binding assay of [^{125}I]*ICYP*. This assay was carried out as described previously [15, 16] with minor modifications. Briefly, the procedure was as follows: The incubation mixture consisted of 0.2 ml of glomerular or tubular particulate suspension (20–40 μg protein/tube), an appropriate concentration of [^{125}I]ICYP, and sufficient 50 mM Tris–HCl, 1 mM EGTA buffer (pH 7.4 at 25°) to make the final incubation volume 0.3 ml. In the competitive binding studies using agonists, the incubation medium in addition contained 10 mM magnesium chloride and 0.8 mM ascorbic acid. Incubations were carried out in 13 × 100 mm glass tubes at 37° for 60 min in a Dubnoff metabolic shaker (Precision Scientific Co.) oscillating at 100 oscillations/min (preliminary studies indicated that equilibrium binding occurred at or beyond 40 min of incubation). After the incubation period the reaction mixtures were filtered under reduced pressure through Whatman glass fiber filters (GF/B). The automatic cell harvester for receptor binding assays from the Brandel Corp. (Gaithersburg, MD) was used for this purpose. The filters were rinsed with 16 ml of ice-cold Tris–EGTA buffer to remove most of the unbound radioactive ligand. They were then dried and placed in 12 × 75 mm plastic tubes and counted in a Packard Prias gamma scintillation counter at 57% efficiency. Specific binding was defined as the difference between binding of the radioligand (determined in duplicate for each concentration) in the absence and presence of 1 μM (−)alprenolol or 100 μM (−)isoproterenol bitartrate, in the competition binding studies.

Data analysis. In experiments requiring the determination of K_D, the dissociation constant, and B_{max}, the maximal number of binding sites, the method of Scatchard [17] was used. The line of the Scatchard plot was determined by linear regression, and the coefficient of linear regression (r) was 0.95 or higher in all cases. In calculating binding parameters, ICYP was assumed to undergo molecular decomposition into non β-receptor active products in direct relation to radioactive decay [18].

For determination of β_1- and β_2-subtypes in glomeruli and tubules, competition experiments were per-

formed using the β_1-selective agent betaxolol to compete for [^{125}I]ICYP binding. The competition curves obtained were analyzed by computer using an iterative, nonlinear curve-fitting program LIGAND [19] adapted for use on microcomputers [20]. This program fits the binding data to equations describing the laws of mass action for one and two classes of binding sites and determines whether the fit for the two-site model is statistically better than that for the one-site model. The relative proportions of each of the two sites (when applicable) and the affinities of the competing agent for the two sites are provided by the output of the program.

Adenylate cyclase activity measurements. This assay was carried out as described previously [16]. Briefly, the procedure was as follows: The standard reaction mixture contained 50 mM Tris–HCl (pH 7.4; 30°), 0.3 mM EGTA, 10 mM theophylline, 2 mM $MgCl_2$, 5 mM phosphocreatine, 10 μM GTP, 15 units creatine phosphokinase, and 1 mM ATP. The assay tubes (13 × 100 mm glass tubes) containing the reaction mixture were preincubated for 5 min at 30° in a Dubnoff metabolic shaker at 100 oscillations/min. Reaction was started by the addition of 75–150 μg of membrane protein in 100 μl volume. The final reaction volume was 0.5 ml. In experiments measuring hormone-stimulated adenylate cyclase activity, hormone was part of the preincubation mixture. Incubation was carried out for 15 min at 30°, and the reaction was stopped by boiling the tubes for 3 min. After centrifugation at 2000 *g* for 15 min at 4°, aliquots of supernatant fluid were assayed for cAMP by the method of Gilman [21] using the [^{3}H]cAMP assay kit obtained from the Diagnostic Products Corp. Under these conditions, cAMP accumulation was linear for at least 15 min. All assays were carried out in duplicate.

Results obtained were expressed as picomoles cAMP accumulated per milligram protein per minute. Values of hormone-stimulated cAMP accumulation were obtained by subtracting the basal values obtained in the absence of hormone from the values obtained in the presence of hormone.

Membrane protein measurement. Membrane protein was estimated by the method of Lowry *et al.* [22].

RESULTS

Saturability and affinity of [^{125}I]*ICYP binding.* The specific binding of [^{125}I]ICYP was saturable with increasing ligand concentration in both glomeruli and tubules (Fig. 2). Scatchard analysis of saturation isotherms yielded linear plots indicating that [^{125}I]ICYP recognized a single class of noncooperative binding sites (Fig. 2, inserts). In both glomeruli and tubules, [^{125}I]ICYP showed very high binding affinity. The values of K_D from eight experiments were respectively (mean ± SE): glomeruli, 9.2 ± 1.3; tubules, 9.7 ± 0.6 pM. The B_{max} values were about three times as high in glomeruli as in tubules (Fig. 2). Typically, in the experiment, specific binding values, using a concentration around K_D and with about 30 μg of membrane protein per tube, ranged around 6000 cpm for the glomeruli and 2000 cpm for the tubules. At concentrations around K_D, the nonspecific binding was relatively low, constituting 10–20% of total binding. The aqueous blank was less than 3%.

Stereospecificity of [^{125}I]*ICYP binding.* The binding of [^{125}I]ICYP to glomerular and tubular particulate fractions showed the stereospecificity expected of binding occurring to β-adrenergic receptors (Fig. 3). (−)Isoproterenol was 10–35 times more potent than (+)isoproterenol in competing for [^{125}I]ICYP binding.

Agonist potency in competing for [^{125}I]*ICYP binding.* In both glomeruli and tubules, (−)isoproterenol was more potent than (−)epinephrine or (−)norepinephrine in competing for [^{125}I]ICYP binding, as would be expected of β-adrenergic receptor binding sites (Fig. 4). (−)Epinephrine and (−)norepinephrine were about equipotent, indicating that the binding was predominantly of the β_1-subtype.

Relative amounts of β_1 and β_2-adrenoceptors. To determine the relative percentages of β_1- and β_2-adrenoceptors in glomeruli and tubules, competition experiments were performed using the β_1-selective antagonist betaxolol to compete for [^{125}I]ICYP binding. Data obtained were analyzed by computer

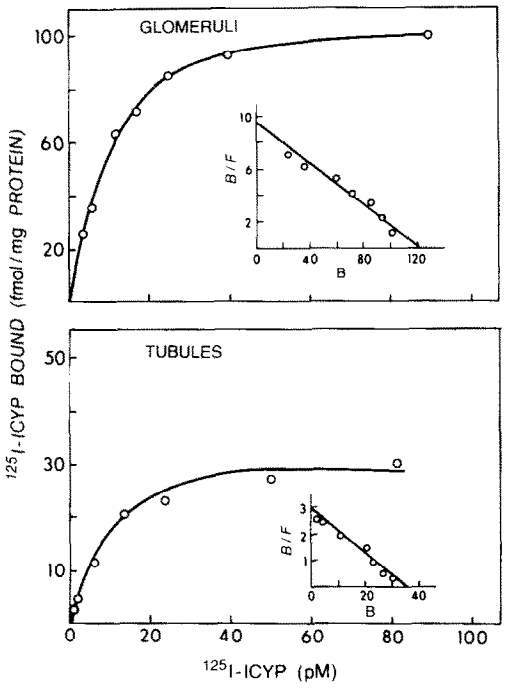

Fig. 2. Saturation of [^{125}I]ICYP binding with increasing ligand concentration in kidney glomeruli and tubules. Particulate fractions were incubated with increasing concentrations of [^{125}I]ICYP at 37° for 60 min. Specific binding was determined as described under Materials and Methods. Results shown are those obtained in a typical experiment using pooled samples from two to three rats. Inserts show Scatchard plots generated from the corresponding specific binding data using linear regression analysis. The values of B_{max} calculated from eight different experiments each using pooled samples from two to three rats were (mean ± SE): glomeruli, 111.1 ± 8.9; and tubules, 40.1 ± 1.8 fmol/mg protein. The corresponding K_D values were: glomeruli, 9.2 ± 1.3; and tubules 9.7 ± 0.6 pM.

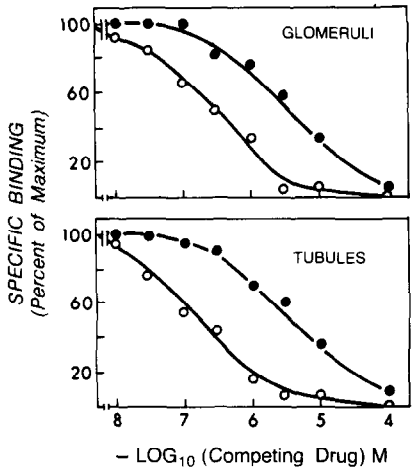

Fig. 3. Stereoselectivity of agonist competition for [^{125}I]ICYP binding in glomeruli and tubules. Particulate fractions were incubated with [^{125}I]ICYP (30 pM) and increasing concentrations of (−)isoproterenol (○—○) and (+)isoproterenol (●—●). For calculation of specific binding, binding occurring in the presence of 100 μM (−)isoproterenol was taken to represent nonspecific binding. The data shown are representative of those obtained in three similar experiments each using pooled tissue from two to three rats.

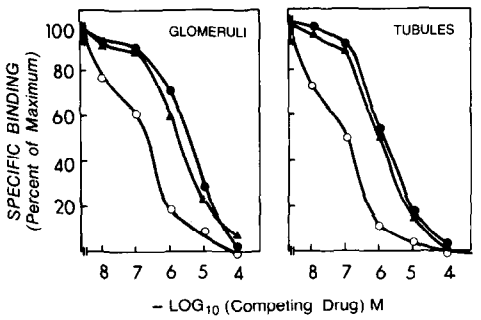

Fig. 4. Rank order of potency of competition by agonists for [^{125}I]ICYP binding in glomeruli and tubules. Particulate fractions were incubated with [^{125}I]ICYP (30 pM) and increasing concentrations of indicated compounds: (−)isoproterenol (○—○); (−)norepinephrine (●—●); and (−)epinephrine (▲—▲). For specific binding determination, binding occurring in the presence of 100 μM (−)isoproterenol was taken to represent nonspecific binding. The data shown are representative of those obtained in three similar experiments each using pooled tissue from two to three rats.

using the LIGAND program. In both glomeruli and tubules, betaxolol competition for [^{125}I]ICYP yielded curves with slopes significantly less than unity, as shown in Table 1. In all the experiments, the two-site fit was significantly better than the one-site fit. The β_1-subtype was the predominant subtype of β-adrenoceptor in the glomeruli and tubules constituting more than 80% of the total β-adrenoceptors (Table 1). There was no significant difference in the proportion of β_1- and β_2-subtypes between the glomeruli and tubules. The affinities of betaxolol for the β_1- and β_2-subtypes were similar in the glomeruli and tubules.

Isoproterenol competition for [^{125}I]ICYP binding and guanyl nucleotide effect on the competition. The observation that glomeruli had three times the concentration of β-adrenoceptors as the tubules made it of interest to investigate whether an agonist such as (−)isoproterenol competed for [^{125}I]ICYP specific binding similarly in the glomeruli and tubules and whether guanyl nucleotides affected the (−)isoproterenol competition curve similarly in the two tissues. Since both β_1- and β_2-adrenoceptor subtypes are present in the glomeruli and tubules, an agonist (such as isoproterenol) recognizes high and low affinity forms of each receptor subtype in these tissues resulting in a complex interaction involving four binding sites [23]. None of the currently available techniques can satisfactorily analyze data involving binding to more than two or three classes of sites. However, since the glomeruli and tubules have similar proportions of β_1- and β_2-subtypes (Table 1), a useful comparison between the two tissues can be obtained by defining the IC_{50} values of isoproterenol in the two tissues in the absence and presence of Gpp(NH)p. Results of experiments carried out to define these values are shown in Fig. 5.

Isoproterenol was about twice as potent in competing for the [^{125}I]ICYP binding in tubules as in the glomeruli (Fig. 5, Table 2), and this difference was statistically significant ($P < 0.05$). Gpp(NH)p caused a rightward shift (i.e. a decrease in affinity) of isoproterenol competition curve in both the glomeruli and tubules (Fig. 5). The extent of the shift (-fold shift) of the isoproterenol competition curve caused by Gpp(NH)p in the glomeruli and tubules was similar and was about 3-fold (Table 2).

The data of isoproterenol competition for [^{125}I]ICYP binding and the Gpp(NH)p effect on this were analyzed further by generation of pseudo-Hill plots (Fig. 6). The isoproterenol competition curve in the absence of Gpp(NH)p exhibited a slope factor (pseudo-Hill coefficient) of 0.74 ± 0.04 in the glom-

Table 1. Competition for [^{125}I]ICYP binding by betaxolol in glomeruli and tubules

Group	Slope factor	% β_1	% β_2	$K\beta_1$ (nM)	$K\beta_2$ (nM)
Glomeruli	0.80 ± 0.01	89.0 ± 1.9	11.0 ± 1.9	11.3 ± 0.8	430 ± 114.5
Tubules	0.73 ± 0.04	83.0 ± 1.9	17.0 ± 1.9	12.5 ± 1.2	445 ± 153.2

Particulate fractions were incubated with [^{125}I]ICYP and various concentrations of the β_1-selective antagonist betaxolol. Data were analyzed by computer as described under Materials and Methods. Values shown are means ± SE of four different experiments each using pooled tissue from two to three rats.

Table 2. Comparison of isoproterenol competition for [^{125}I]ICYP binding and isoproterenol stimulation of cAMP accumulation in the glomeruli and tubules

Group	IC_{50} (nM)			Slope factor		EC_{50} for Isoproterenol-stimulated cAMP accumulation (nM)
	−Gpp(NH)p	+Gpp(NH)p	−Fold shift	−Gpp(NH)p	+Gpp(NH)p	
Glomeruli	325 ± 37	985 ± 76	3.3 ± 0.3	0.74 ± 0.04	0.94 ± 0.03	114 ± 13
Tubules	163 ± 18*	455 ± 14*	3.1 ± 0.4	0.54 ± 0.02*	0.83 ± 0.04*	19 ± 3*

For IC_{50} determinations, curves of (−)isoproterenol competition for [^{125}I]ICYP specific binding were determined in the presence and absence of Gpp(NH)p as described in the legend to Fig. 5, and concentrations of (−)isoproterenol producing 50% inhibition were determined from the curves. The degree of shift (−fold shift) was determined from paired experiments as the ratio of the IC_{50} values obtained in the presence and absence of 100 μM Gpp(NH)p. Slope factors were calculated from pseudo-Hill plots generated from (−)isoproterenol competition curves as described in the legend to Fig. 6. The concentrations of isoproterenol producing 50% of maximal cAMP accumulation (EC_{50}) were calculated from concentration–effect curves generated as described in the legend to Fig. 7. Results shown are means ± SE of eight different experiments each using pooled tissue from two to three rats.

* $P < 0.05$ compared to corresponding value for glomeruli by Student's *t*-test.

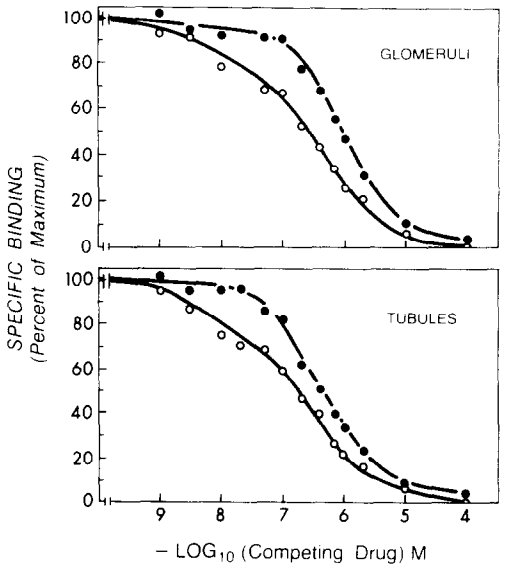

Fig. 5. Effect of Gpp(NH)p on competition by (−)isoproterenol for [^{125}I]ICYP specific binding sites in the glomeruli and tubules. Particulate fractions were incubated with 25 pM [^{125}I]ICYP and various concentrations of (−)isoproterenol in the absence (○—○) and presence (●—●) of 100 μM Gpp(NH)p. Values obtained were expressed as percent of the maximal specific binding, which was the binding occurring in the absence of isoproterenol or Gpp(NH)p. In these experiments, binding occurring in the presence of 100 μM (−)isoproterenol was taken to represent nonspecific binding. Results shown are representative of those obtained in eight similar experiments each using pooled tissue from two to three rats.

eruli and 0.54 ± 0.02 in the tubules (Table 2), and this difference was statistically significant ($P < 0.05$). In the presence of Gpp(NH)p, there was a steepening of the curve and an increase in the slope factor in both cases (Table 2), the magnitude of the change being somewhat higher in the tubules as compared to glomeruli; however, the difference between the slope factors in the glomeruli and tubules in the presence of Gpp(NH)p still remained significant ($P < 0.05$).

Beta-adrenergic stimulated adenylate cyclase activity in the glomeruli and tubules. Basal and isoproterenol-stimulated adenylate cyclase activities were measured in glomeruli and tubules. The results obtained are shown in Fig. 7. The basal activity was higher in the tubules than in the glomeruli. Isoproterenol stimulation of cAMP accumulation was of a much higher magnitude in the tubules than in the glomeruli at all concentrations of isoproterenol used. This was true whether the increase was measured in terms of actual picomoles per milligram per minute of cAMP accumulated or in terms of percent increase over basal activity, thus taking into consideration any existing differences in the basal activities in the two tissues (Fig. 7). The concentration of isoproterenol producing half-maximal stimulation (EC_{50}) was 114 ± 13 nM in the glomeruli and 19 ± 3 nM in the tubules (Table 2), and this difference was statistically significant ($P < 0.05$).

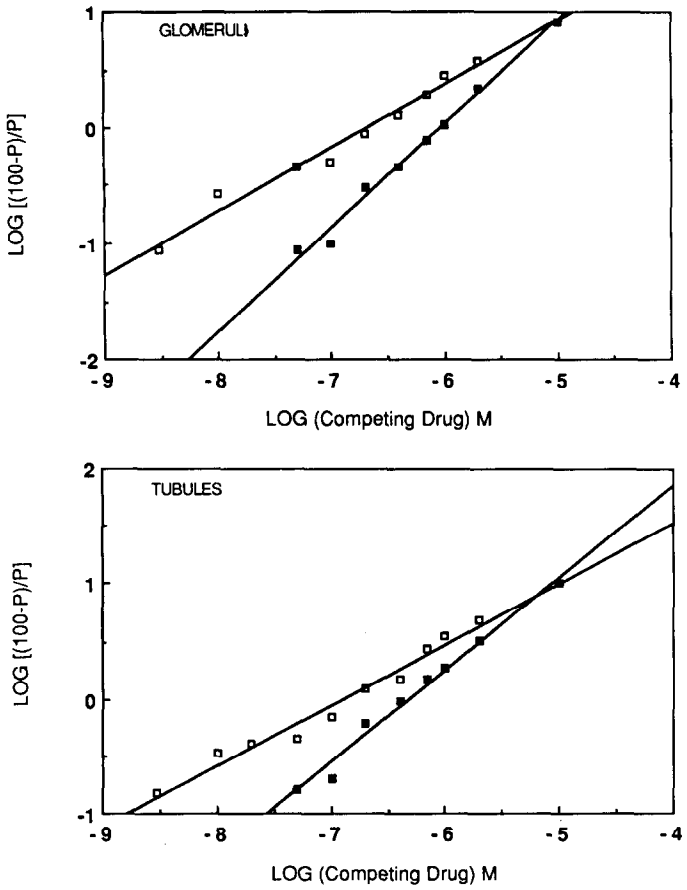

Fig. 6. Pseudo-Hill plot of (−)isoproterenol competition for [^{125}I]ICYP specific binding sites in the absence and presence of Gpp(NH)p in the glomeruli and tubules. The pseudo-Hill plots shown were generated from the isoproterenol competition curves shown in Fig. 5. Only values between 10 and 90% inhibition of specific binding were included in the analysis. Plots shown are those obtained in the absence (□—□) and presence (■—■) of 100 μM Gpp(NH)p. In the ordinate, P represents specific binding expressed as percent of maximum. Results shown are representative of those obtained in eight similar experiments each using pooled tissue from two to three rats.

Stimulation of adenylate cyclase in the glomeruli and tubules by other hormones, Gpp(NH)p and forskolin. In view of the difference in the effectiveness of isoproterenol in causing cAMP accumulation in the glomeruli and tubules, the effects of other hormones and agents that activate adenylate cyclase in the glomeruli and tubules were studied. Results of these studies are shown in Table 3.

PTH and AVP both stimulated adenylate cyclase in the glomeruli and tubules and, as expected, were

Table 3. Comparison of PTH, AVP, Gpp(NH)p and forskolin stimulation of cAMP accumulation in the glomeruli and tubules

	cAMP Accumulation (% stimulation over basal)				
Group	PTH (5 μg/ml)	AVP (1 unit/ml)	Gpp(NH)p (1 μM)	Gpp(NH)p (100 μM)	Forskolin (10 μM)
Glomeruli	51.2 ± 4.1	11.7 ± 2.2	28.4 ± 4.6	155 ± 20.5	180.8 ± 34.7
Tubules	160.8 ± 11.6*	60.3 ± 4.6*	25.7 ± 3.3	174.8 ± 7.8	216.7 ± 3.0

Tissue preparation and assay conditions are described in Materials and Methods. For obtaining "percentage stimulation over basal" values, the basal values were subtracted from the values obtained in the presence of the stimulating agent, and the results were expressed as a percentage of the basal. Basal values of cAMP accumulation were: glomeruli, 7.9 ± 0.6; and tubules, 12.4 ± 0.9 fmol/mg protein/min. Values are means ± SE of four different experiments each using pooled tissue from two to three rats in all groups except the forskolin group where there were six different experiments.

* $P < 0.05$ compared to the corresponding value for the glomeruli by Student's *t*-test.

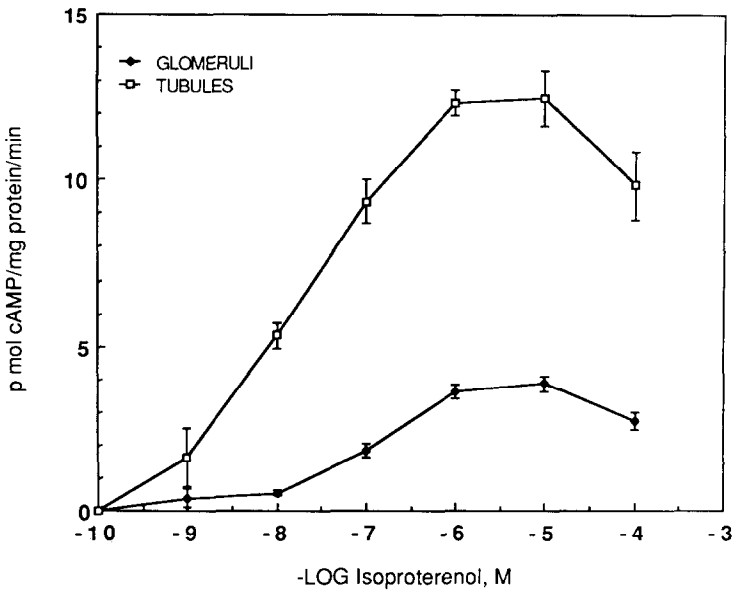

Fig. 7. Concentration–effect curves for (−)isoproterenol-stimulated cAMP accumulation in the glomeruli and tubules. Tissue preparation and assay conditions are described in Materials and Methods. Values of isoproterenol-stimulated cAMP accumulation were calculated by subtracting the basal values from those obtained in the presence of (−)isoproterenol. Basal values were: glomeruli, 7.8 ± 1.3; and tubules, 13.3 ± 2.0 fmol/mg protein. Values shown are means ± SE of four different experiments each using pooled tissue from two to three rats.

much more potent in the tubules than in the glomeruli (Table 3). The effectiveness of both PTH and AVP in stimulating adenylate cyclase in the tubules indicated that both proximal and distal tubular segments were present in the tubular preparation used in the current study.

The effects of agents which bypass the receptor and stimulate cAMP formation by acting on the guanine nucleotide binding protein (N_s) and/or the catalytic site (C) of the adenylate cyclase were of special interest. The stimulation by Gpp(NH)p, which directly acts on N_s [24], and by forskolin, the plant-derived terpene which produces a major portion of its effect by acting on the catalytic unit [25], in the glomeruli and tubules is shown in Table 3. The percentage stimulation of cAMP accumulation over basal in response to Gpp(NH)p and forskolin was somewhat higher in the tubules as compared to the glomeruli, but the difference was not statistically significant at the $P < 0.05$ level.

DISCUSSION

The use of [^{125}I]ICYP to identify and characterize β-adrenoceptors in whole renal cortical preparations has been reported by other workers [10, 26]. The results of our study indicated that [^{125}I]ICYP is also an excellent radioligand for characterization and quantification of β-adrenergic receptors in isolated renal glomeruli and tubules. [^{125}I]ICYP as a radioligand offers many advantages: high affinity, high specific activity, low nonspecific binding, and excellent stability. Perhaps the greatest advantage offered by [^{125}I]ICYP for binding studies in the case of isolated glomeruli and tubules is the small amount of membrane protein required for the studies. Thus, very satisfactory specific binding values were obtained in the study with as little as 20 μg of membrane protein per tube, and Scatchard plots with six or seven different concentrations could be generated from tissues obtained from two to three rats. In contrast, when [^{3}H]dihydroalprenolol is used as radioligand, approximately 500 μg/tube of membrane protein are required, and tissue may need to be pooled from as many as ten rats for generating Scatchard plots [11, 12].

Although most previous adrenergic radioligand binding studies in the kidney have used whole renal cortical membrane preparations, a few studies have been reported using isolated tubules and glomeruli. Gavendo *et al.* [11] reported on [^{3}H]dihydroalprenolol binding to rat kidney tubules. Their B_{max} value (50.3 ± 4.5 fmol/mg protein; mean ± SE; N = 7) was in the same range as found in the current study. The K_D value was in the nanomolar range (mean K_D value was 7.1 nM), indicating the much lower affinity of [^{3}H]dihydroalprenolol for kidney β-adrenoceptors as compared to [^{125}I]ICYP. McPherson and Summers [12] have reported on [^{3}H]dihydroalprenolol binding to glomerular enriched fraction from rat kidney. They found that glomerular enriched fraction has four times the density of β-adrenoceptor sites as the whole renal cortex preparation. The results in our study indicating that the B_{max} in the glomeruli was three times that in the tubules is consistent with the above findings. The actual B_{max} value for the glomeruli found in the study of McPherson and Summers [12] was relatively high (mean value 280 fmol/mg protein) as compared to the value found in the present study. The reason for this discrepancy is not clear. In one other study, an apparent negative cooperativity in the binding of

[^{3}H]dihydroalprenolol to rat kidney β-adrenergic receptors has been found [9]. If this is true, it is possible that this factor may have contributed to the overestimation of the B_{max} in the study of McPherson and Summers [12]. Results of the present study agree well with the findings of recent autoradiographic studies of β-adrenoceptors and their subtypes in rat kidney [27–29]. In these studies higher concentrations of β-adrenoceptors were found to be associated with glomeruli and the distal tubules. The receptors in the cortex were found to be predominantly of the β_1-subtype.

Important differences between glomeruli and tubules in their β-adrenoceptor number and β-adrenoceptor-adenylate cyclase coupling are revealed by the present study. The β-adrenoceptor number was three times as high in the glomeruli as compared to the tubules (Fig. 2). However, the β-adrenoceptor-stimulated adenylate cyclase activity (whether it was expressed in terms of percentage increase over basal or increase in terms of actual picomoles per milligram protein) was more than twice in tubules as compared to glomeruli (Fig. 7). Some other results obtained in the study suggest at least a partial explanation for this discrepancy. Thus, isoproterenol was twice as potent in competing for [^{125}I]ICYP binding in the tubules as in the glomeruli (Fig. 5; Table 2), and the slope factor of isoproterenol competition curve in the tubules was significantly less than that in the glomeruli (Fig. 6; Table 2). The extent of shift of the isoproterenol competition curve (-fold shift) produced by Gpp(NH)p was similar in the glomeruli and the tubules (Fig. 6; Table 2). It is generally accepted that the efficacy of the agonist to compete for the antagonist binding, the shallowness of the competition curve as revealed by the low value of the slope factor, and the extent of shift of the agonist competition curve produced by guanyl nucleotides all are indicative of the interaction of the agonist hormone (H), receptor (R) and guanine nucleotide regulatory protein (N) [7]. Thus, overall our results suggest that, in the case of isoproterenol, there is less efficacious H–R–N interaction in the glomeruli as compared to the tubules. This would be expected to translate into less biological effect such as accumulation of cAMP.

The EC_{50} of isoproterenol for causing cAMP accumulation was six times less in the tubules as compared to the glomeruli, indicating once again that isoproterenol was more efficacious in the tubules as compared to the glomeruli. The nucleotide regulatory protein-catalytic site interaction (N–C interaction) as tested by Gpp(NH)p was similar in the glomeruli and tubules (Table 3). Catalytic site (C) activity, as tested at least partly by forskolin, was somewhat less in the glomeruli as compared to the tubules, but the difference was not statistically significant (Table 3). Thus, it would seem that the decreased H–R–N interaction in the glomeruli as compared to the tubules was the primary contributing factor causing the decreased efficacy of isoproterenol in producing cAMP accumulation in the glomeruli as compared to the tubules.

Some other factors may also be involved in causing the apparent decreased efficacy of isoproterenol in the glomeruli as compared to the tubules. Thus, it is possible that in the glomeruli the coupling between the β-adrenoceptor and the adenylate cyclase may be loose, so that partial uncoupling occurs during the preparative procedure. Such poor coupling has been reported for some other tissues like the brain [30]. Somewhat opposed to this possibility is that similar isoproterenol-stimulated cAMP accumulation was found by Kotake *et al.* [31] whether the studies were done on intact glomeruli or on glomerular homogenate preparations. Also the studies of Abboud *et al.* [32] were done with intact glomeruli, whereas our and the the studies of Felder *et al.* [33] were done using glomerular membrane preparation and, in all cases, similar isoproterenol stimulations over basal adenylate cyclase activities were observed. Another possible explanation for the observed findings is that a major portion of the β-adrenoceptors in the kidney glomeruli may not be coupled to adenylate cyclase, and coupling with adenylate cyclase may not be the only mode of receptor–effector coupling for the β-adrenoceptors in the glomeruli. Such observations have been made in some other β-adrenoceptor systems. Waelbroeck *et al.* [34] found that, in the human heart, up to 50% of the β-adrenoceptors may not be coupled to adenylate cyclase. In S49 lymphoma cells, the inhibition of magnesium transport by β-adrenergic agonists acting through β-adrenoceptors has been found to be independent of adenylate cyclase activation [35]. This aspect may be worth evaluating in future experiments on kidney glomeruli.

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