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Renal *Alpha*-1 and *Alpha*-2 Adrenergic Receptors: Biochemical and Pharmacological Correlations[1]

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ABSTRACT

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[^{3}H]Dihydroergocryptine, a nonselective *alpha* adrenergic antagonist, the *alpha*-1 selective antagonist, [^{3}H]prazosin and the *alpha*-2 selective antagonist, [^{3}H]yohimbine, were used to study binding sites in rat renal membranes. To establish a correlation between binding and a biological function, the ability of *alpha* adrenergic agents to stimulate or inhibit vasoconstriction was quantified *in vitro* using an isolated perfused kidney preparation. Binding with each radioligand was rapid, saturable and specific. Moreover, the order of potencies of a variety of adrenergic agents, determined by competitive inhibition studies, suggested that the binding of each radioligand was to sites with *alpha* adrenergic specificity. The total number of binding sites in these rat renal membranes, determined with [^{3}H]dihydroergocryptine (B_{max}, 212 fmol/mg of protein; K_D, 12.8 nM) was approximately equal to the sum of binding site concentrations determined with the *alpha*-1 and *alpha*-2 selective radioligands (B_{max}, 57 and 170 fmol/mg of protein; K_D, 0.85 and 20 nM, respectively). However, the *alpha* receptor mediating renal arteriolar vasoconstriction appeared to be of the *alpha*-1 subtype as there was a close correlation between the *in vitro* results and the binding data determined with [^{3}H] prazosin ($r = 0.93$). In addition, in both the functional and [^{3}H] prazosin binding studies, unlabeled prazosin was 5 to 40-fold more potent than the nonselective antagonist, phentolamine, and 400- to 1500-fold more potent than the *alpha*-2 antagonist, yohimbine. These studies suggest that rat renal plasma membranes contain binding sites with both *alpha*-1 and *alpha*-2 adrenergic receptor specificity, in a ratio of approximately 1:3. Despite the preponderance of *alpha*-2 receptors, the *alpha* receptor mediating renal vasoconstriction appears to be of the *alpha*-1 type.

The regulation of vascular tone in the kidney by the sympathetic nervous system is mediated by *alpha* adrenergic receptors (Baum, 1977). Thus, stimulation of renal nerves decreases renal blood flow (McGiff and Aviado, 1961), whereas the *alpha* adrenergic agonists, epinephrine and norepinephrine, produce dose-dependent decreases in renal blood flow when injected into the renal artery (Balint and Chatel, 1967). More recently, physiological studies have suggested the existence of *alpha* receptors on proximal tubules which mediate sodium reabsorption (Slick *et al.*, 1975; Benesath *et al.*, 1972; Gottschalk, 1979), whereas pharmacological studies suggest that renal *alpha* receptors may also be involved in the control of renin release (Pettinger *et al.*, 1976). However, due to our lack of knowledge of the postreceptor events associated with *alpha* receptor activation, the molecular mechanisms governing these responses are still poorly understood. In the present study, we have thus characterized renal *alpha* adrenergic receptors by the binding of radioligands to rat renal membranes. In addition, the potential for these biochemical studies to quantitatively assess a receptor-coupled response (renal vasoconstriction) was also investigated.

Methods

Radioligand binding studies. Renal plasma membranes were prepared and binding studies were performed generally as described by Williams *et al.* (1976) using [^{3}H]dihydroergocryptine (0.1–40 nM), [^{3}H] prazosin (0.1–6 nM) or [^{3}H]yohimbine (0.1–40 nM). Renal membranes (100 μl, protein concentration 3–4 mg/ml) prepared from both kidneys of adult, male Sprague-Dawley rats (250–360 g b.w.) were incubated with the radioligands for 30 min at 25°C in a buffer containing either 50 mM Tris (pH 7.5) and 10 mM $MgCl_2$ ([^{3}H]dihydroergocryptine and [^{3}H]prazosin) or 50 mM Na_2HPO_4 and 50 mM KH_2PO_4, pH 7.4, ([^{3}H] yohimbine) in a final volume of 150 μl. At the end of the incubation, samples were diluted with 5 ml of the above buffers at 4°C and were rapidly filtered through Whatman GF/C filters. The filters were then washed with three additional 5-ml aliquots of buffer (4°C), dried, placed in scintillation vials and counted in 10 ml of Triton-toluene aqueous scintillation mixture. Counting efficiency was 38%. Specific binding in these studies refers to that fraction of bound radioligand inhibited by 10 μM phentolamine, a concentration we found to be optimal for each

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of the radioligands. Higher concentrations of phentolamine displaced more radioligand but were not utilized because Scatchard (1949) analysis indicated that this additional displacement was from low affinity, high capacity binding sites (Chamness and McGuire, 1975). For use in the assays, the radioligands were diluted to the appropriate concentrations in 10% ethanol, 5 mM HCl and 0.2% bovine serum albumin. This diluent did not alter the pH of the incubation mixture or the binding of the radioligands to the renal membranes, but was effective in decreasing the nonspecific binding of the compounds to the plastic tubes used in the assay. Specific binding represented 70 to 80% of total binding for [^{3}H]prazosin, 55 to 65% for [^{3}H]dihydroergocryptine and 65 to 70% for [^{3}H]yohimbine. Specific binding was not corrected for nonspecific binding to the glass-fiber filters as this was negligible (<0.5%). All experiments were performed in duplicate or triplicate, and the results represent the mean values of separate studies performed in membrane preparations from both kidneys of 4 to 10 different rats. Membrane protein was determined according to the procedure of Lowry *et al.* (1951) using bovine serum albumin as the standard.

The ability of various agonists and antagonists to compete for [^{3}H] dihydroergocryptine, [^{3}H]prazosin or [^{3}H]yohimbine binding to rat renal membranes was also determined. The membranes were incubated with [^{3}H]dihydroergocryptine (6–7 nM), [^{3}H]prazosin (0.4–0.6 nM) or [^{3}H]yohimbine (18–20 nM) for 30 min at 25°C in the presence of increasing concentrations of the various agonists and antagonists shown in table 1. The apparent dissociation constants (K_D) for these agents were then calculated from the equation:

$$K_D = \frac{IC_{50}}{1 + \frac{[S]}{K_D}}$$

(Williams *et al.*, 1976), where IC_{50} is the concentration of an agent giving 50% inhibition of radioligand binding, S represents the concentration of radioligand and K_D is the dissociation constant of [^{3}H] dihydroergocryptine (7.5 nM), [^{3}H]prazosin (0.4 nM) or [^{3}H]yohimbine (15.5 nM) as determined by equilibrium binding and kinetic studies. The K_D values of the drugs shown in table 1, determined in competitive inhibition studies, are the mean values obtained from separate experiments in membrane preparations from both kidneys of four to six different rats per drug.

To investigate whether the binding of the radioligands was to pre- or postjunctional receptors, additional [^{3}H]dihydroergocryptine binding studies were performed using membranes prepared from the kidneys of rats sympathectomized with 6-hydroxydopamine (66 mg/kg i.v., administered both 7 days and 24 hr before removal of the kidneys). In these studies, the effectiveness of chemical sympathectomy was confirmed by the finding that vasoconstrictor responses to periarterial nerve stimulation were completely abolished by 6-hydroxydopamine pretreatment. In contrast, electrically elicited vasoconstriction was readily demonstrated in kidneys from untreated rats.

Studies in the isolated perfused kidney. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), the left renal vessels were exposed anteriorly by a midline incision and sodium heparin (600 U i.v.) was administered. Two minutes thereafter the suprarenal aorta was clamped and all remaining blood was flushed from the kidney with a modified Krebs' solution (pH 7.4, osmolarity 284 mOsm/kg of H_2O) of the following composition (in millimolars): NaCl, 112; KCl, 5.0; NaH_2PO_4, 1.0; $MgCl_2$, 0.5; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; D-(+)-glucose, 11.2; pyruvate, 2.0; glutamate, 2.6; and Dextran, 2%. The kidney was then rapidly removed and, after cannulation of the renal artery with polyethylene tubing (PE 50), suspended in a tissue chamber and perfused at a constant flow of 2 ml/min with a Harvard model 1210 peristaltic pump (Harvard Apparatus, Mills, MA). The perfusate was maintained at 25°C and was aerated with a 95% O_2-5% CO_2 mixture. Changes in perfusion pressure were monitored by a Narco RP-1500 pressure transducer (Narco Scientific Industries, Inc., Fort Washington, PA) and recorded on a Grass model 7 polygraph (Grass Instrument Co., Quincy, MA). The average mean pressure in the cannula, before cannulation of the renal artery, was 2.0 ± 0.1 (S.E.M.) mm Hg (n = 10), whereas the average mean pressure after cannulation and a 20-min stabilization period was 48.6 ± 3.5 mm Hg (n = 10). Under these conditions, basal pressure remained constant throughout the course of an experiment (<2 hr). Test drugs were administered by constant infusion (Harvard infusion pump, model 975) at a rate of 0.05 ml/min *via* an infusion port proximal to the peristaltic pump. All drugs were dissolved in distilled water according to their molecular weights and diluted to deliver a semilog distribution of concentrations. As the perfusate flow was constant, changes in renal vascular resistance were quantified by the changes in perfusion pressure.

Dose-response curves of the agonists listed in table I were quantitated in the isolated perfused kidney by determining the steady-state

TABLE 1

Apparent dissociation constants (K_D) and potency ratios for adrenergic agents determined in the isolated perfused kidney and in competitive inhibition studies with [^{3}H]prazosin or with [^{3}H]dihydroergocryptine ([^{3}H]DHE) (See text for details)

Drug[a]	Isolated Perfused Kidney		[^{3}H]Prazosin		[^{3}H]DHE	
	K_D	P.R.[b]	K_D	P.R.[b]	K_D	P.R.[b]
	nM		*nM*		*nM*	
−EPI	230	1.0	3,900	1.0	440	1.0
−NE	320	0.72	4,425	0.9	710	0.62
−Phenyl	492	0.47	4,3309	0.9	4,120	0.107
Clonidine	3,000	0.077	3,190	1.2	320	1.38
−αCH_3NE	3,980	0.058	88,500	0.044	2,800	0.157
+NE	7,480	0.031	82,465	0.047	7,300	0.06
+EPI	11,600	0.02	44,300	0.088	4,540	0.0969
Dopamine	>332,000	0.0007	460,000	0.085	8,800	0.05
+αCH_3dopa	>328,000	0.0007	186,500	0.021	18,500	0.024
−ISO	>3,870,000	0.00006	788,000	0.0049	224,000	0.002
DHE	1.02	1.0	12.3	1.0	12.1	1.0
[^{3}H]DHE	1.54	0.7			12.8	0.95
Prazosin	2.25	0.45	0.51	24.0	120	0.1
Phentol	11.5	0.089	22.5	0.55	21	0.576
+Butaclamol	21	0.049	187	0.066	250	0.048
Yohimbine	1,000	0.049	814	0.015	147	0.082
−Butaclamol	1,000	0.001	2,415	0.0051	25,000	0.00048
±Propranolol	20,000	0.000051	30,480	0.0004	30.700	0.00039

[a] Abbreviations: EPI, epinephrine; NE, norepinephrine; Phenyl, phenylephrine; αCH_3NE, α-methylnorepinephrine; ±-αCH_3dopa, ±-α-methyldopamine; ISO, isoproterenol; DHE, dihydroergocryptine; Phentol, phentolamine.

[b] The potency ratio (P.R.) was determined by dividing the K_D value of the reference drugs, epinephrine (agonists) and dihydroergocryptine (antagonists) by that determined for the other agents. This ratio was arbitrarily assigned as 1.0 for the reference drugs.

vasoconstrictor effects of varying doses of each agonist. A single kidney was used to determine an entire dose-response curve for a given agent. The K_D for each agonist was then determined using probit transformation of the dose-response curves (Goldstein *et al.*, 1974). The ability of agonists to inhibit *alpha* adrenergic receptor-mediated vasoconstrictor responses were determined as follow: 1) vasoconstrictor responses to the *alpha* agonist (−)-norepinephrine were established as described above; 2) infusion of an antagonist was started and increasing doses of (−)-norepinephrine were administered until the steady-state vasoconstrictor response was the same as that determined in the absence of the antagonist. The K_D of the antagonist was then quantitated using the Schild equation (Goldstein *et al.*, 1974). The K_D values for the agonists and antagonists shown in table 1 are the mean values obtained in three to four different kidneys per drug.

The drugs used and their source of supply were as follow: (−)-epinephrine, (−)-norepinephrine, dopamine, ergotamine, (±)-propranolol, yohimbine, bradykinin, vasopressin, angiotensin II, substance P and various other chemicals were obtained from Sigma Chemical Company (St. Louis, MO); [^{3}H]dihydroergocryptine was obtained from New England Nuclear (Boston, MA); and [^{3}H]yohimbine from Amersham (Arlington, IL). The following compounds were gifts: [^{3}H]prazosin (Pfizer, Sandwich, Kent, U.K.); dihydroergocryptine and methysergide (Sandoz Pharmaceuticals, E. Hanover, NJ); (±)-α-methyldopamine and (−)-α-methyldopamine (Merck, Sharp and Dohme, Rahway, NJ); nordefrin, levonordefrin, (+)-epinephrine and (+)-norepinephrine (Sterling Winthrop, Rensselaer, NY); (+)-butaclamol and (−)-butaclamol (Ayerst, New York, NY); phentolamine (Ciba-Geigy, Ardsley, NY); clonidine (Boehringer-Ingelheim, Elmsford, NY); prazosin (Pfizer, New York, NY); and haloperidol (McNeil, Fort Washington, PA).

Statistical analyses were performed using the Student's *t* test for unpaired observations, linear regression analysis and analysis of covariance (Zar, 1974).

Results

Radioligand binding studies. Binding of [^{3}H]dihydroergocryptine was time and concentration dependent, saturable and specific (see figs. 1 and 2). Scatchard plots of equilibrium-binding studies (see fig. 1) yielded a straight line. As calculated from the slope of this line and the *X*-intercept, a K_D of 12.8 ± 0.7 (S.E.M.) nM and a binding-site concentration of 212 ± 9 (S.E.M.) fmol/mg of protein, respectively, were obtained. This K_D was in close agreement with the value determined by kinetic analysis:

$$K_D = \frac{k_2}{k_1} = 5.1 \text{ nM}$$

(see fig. 1) and that determined for unlabeled dihydroergocryptine in competition studies (12.1 nM). The *alpha* adrenergic specificity of [^{3}H]dihydroergocryptine binding was suggested by the finding that agonists competed for the binding of the radioligand in the order: clonidine > (−)-epinephrine > (−)-norepinephrine > (−)-α-methylnorepinephrine > dopamine > isoproterenol (see table 1). This order of potency is similar to that observed for rabbit aortic strips (Furchgott, 1972; Besse and Furchgott, 1976), for pressor effects in intact animals (Ahlquist, 1948) and for inhibition of [^{3}H]dihydroergocryptine binding in bovine aortic membranes (Tsai and Lefkowitz, 1978). Stereospecificity of agonist binding was also observed as the affinities of (+)-epinephrine and (+)-norepinephrine were 10-fold less than those of their respective (−)-isomers (table 1). Finally, other vasoactive agents such as bradykinin, angiotensin II, vasopressin and substance P did not inhibit [^{3}H]dihydroergocryptine binding in concentrations ranging from 10^{-9} to 10^{-3} M.

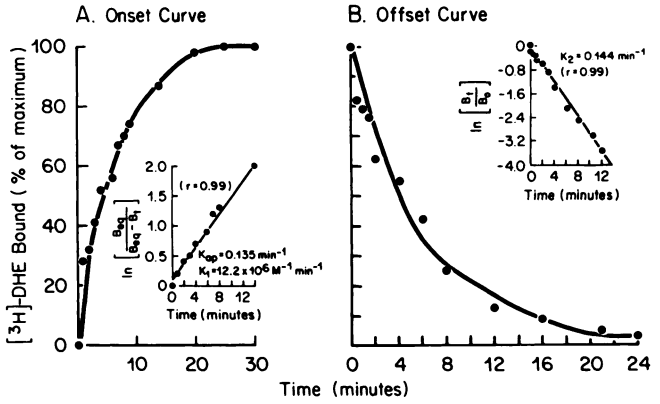

Fig. 1. Kinetic analysis of [^{3}H]dihydroergocryptine ([^{3}H]DHE) binding to rat renal membranes. A, association curve (onset curve) determined by the incubation of [^{3}H]DHE (6–8 nM) with 100-μl membranes in a total volume of 150 μl at 25°C for the times indicated. Results shown are the means of duplicate determinations from an experiment which is representative of four comparable assays. Inset, determination of the second-order rate constant for [^{3}H]DHE binding. B_{eq} is the amount of [^{3}H]DHE bound at equilibrium and B_t is the amount bound at each time, *t*. The slope of the line relating $\ln[B_{eq}/B_{eq} - B_t]$ and time, determined by linear regression analysis (Zar, 1974) is equal to the apparent rate constant for the pseudo-first order reaction of association (K_{ap}). The second order rate constant, k_1, is calculated according to the equation $k_1 = H_{ap} - k_2/([^3H]DHE)$, where k_2 is the dissociation rate constant and ([^{3}H]DHE) the concentration of [^{3}H]DHE used in the assay (Williams *et al.*, 1976). B, dissociation curve (offset curve) determined by the addition of phentolamine to a final concentration of 10 μM after [^{3}H] DHE was first incubated with the membranes for 30 min. At the times indicated, the reaction was stopped by the addition of buffer (see text for details). Inset, first order rate plot of the dissociation of the receptor-ligand complex. The dissociation rate constant, k_2, is equal to the slope of the line relating $\ln[B_t/B_0]$ and time, where B_t refers to the amount of specific binding at each time, *t*, and B_0 is the amount of binding present immediately before the addition of phentolamine.

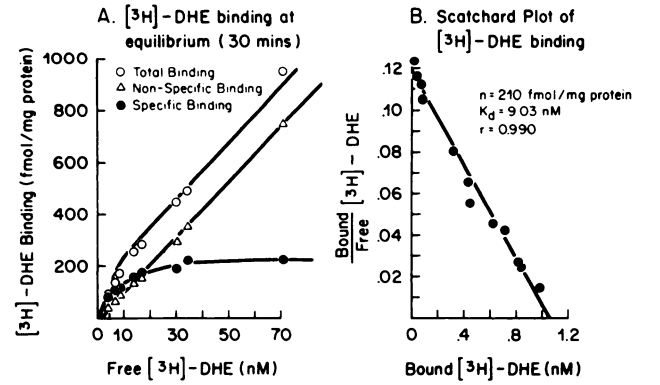

Fig. 2. A, binding (femtomoles per milligram of protein) of various concentrations of [^{3}H]DHE at equilibrium and B, Scatchard analysis of the specific binding data shown in A (see text for details).

Competitive inhibition studies with adrenergic antagonists additionally suggested that [^{3}H]dihydroergocryptine was binding to *alpha* adrenergic receptors as these agents more potently inhibited [^{3}H]dihydroergocryptine binding than did the *beta* adrenergic antagonist, (±)-propranolol, the serotonin antagonist, methysergide, and the dopamine antagonists, (−)-butaclamol and (−)-haloperidol. The active (+)-isomer of the dopaminergic antagonist, butaclamol, competed potently for [^{3}H] dihydroergocryptine binding. However, this agent was also a potent *alpha* antagonist in the isolated perfused kidney (see

table 1), which confirms previous findings that the specificity of this agent for dopamine receptors is low (Quik *et al.*, 1978).

The binding of [^{3}H]yohimbine was rapid, reversible and saturable (see figs. 3 and 4). Equilibrium was achieved within 10 min. Scatchard analysis of the equilibrium-binding studies revealed a K_D of 20 ± 1.1 (S.E.M.) nM and a binding site concentration of 170 ± 10 (S.E.M.) fmol/mg of protein (see fig. 4). This K_D was in agreement with the mean value determined by kinetic analysis (7.4 nM) and that determined for unlabeled yohimbine (19.2 nM) in competitive inhibition studies. The specificity of [^{3}H]yohimbine binding to *alpha*-2 receptors was suggested by the relative order of potencies of *alpha* adrenergic antagonists to displace the ligand in competitive inhibition studies: yohimbine (19.2 nM) > dihydroergocryptine (26.2 nM) > phentolamine (28.5 nM) > prazosin (142.0 nM).

Binding of [^{3}H]prazosin was rapid, reversible and saturable (see figs. 4 and 5). Equilibrium was achieved within 15 min. Scatchard analysis of the equilibrium-binding data revealed a K_D of 0.85 ± 0.05 (S.E.M.) nM and a binding-site concentration of 57 ± 6 (S.E.M.) fmol/mg of protein (see fig. 4). *Alpha*-1 adrenergic specificity and stereospecificity of [^{3}H]prazosin binding is indicated by the following order of potencies of agonists: (−)-epinephrine > (−)-phenylephrine = (−)-norepinephrine > (+)-norepinephrine > (−)-α-methylnorepinephrine > (±)-methyldopamine > (−)-dopamine > isoproterenol and of the antagonists: prazosin > dihydroergocryptine > phentolamine > (+)-butaclamol > yohimbine > (−)-butaclamol > (±)-propranolol.

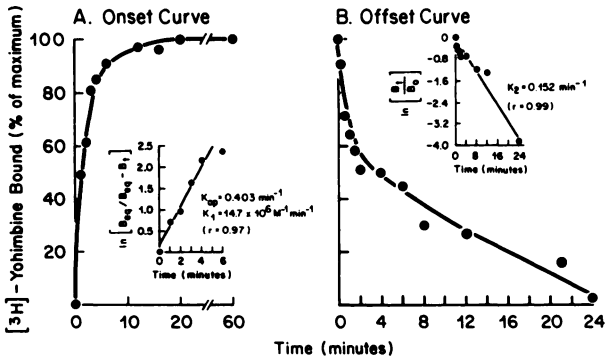

Fig. 3. Kinetic analysis of [^{3}H]yohimbine binding to rat renal membranes. Dissociation (A, onset) and association (B, offset) curves were constructed and rate constants were determined as detailed in the legend to figure 1. Assays were performed using 15 to 20 nM [^{3}H] yohimbine.

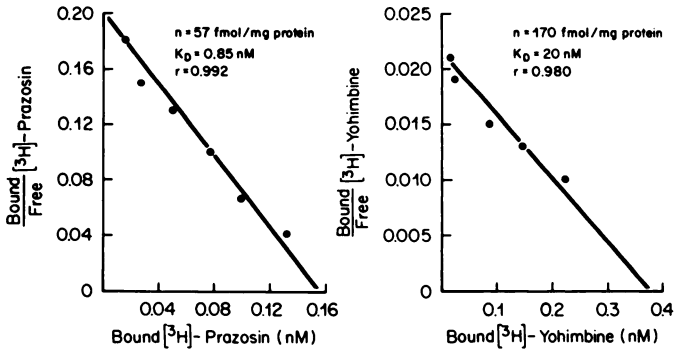

Fig. 4. Scatchard analysis of equilibrium binding studies performed with [^{3}H]prazosin and [^{3}H]yohimbine (see text for details).

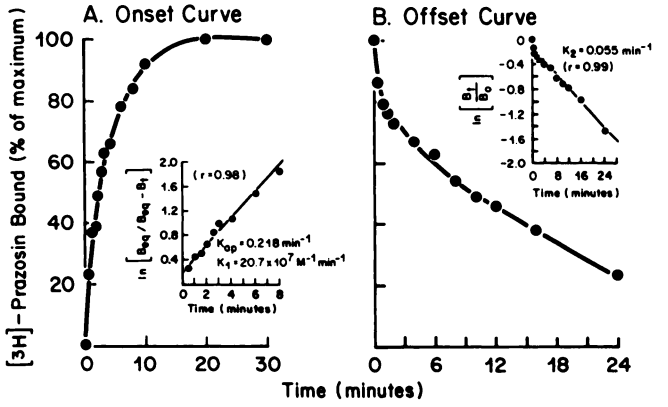

Fig. 5. Kinetic analysis of [^{3}H]prazosin (0.4–0.6 nM) binding to rat renal membranes (see legends to figures 1 and 3 for details).

Renal membranes from 6-hydroxydopamine-pretreated rats bound 212 ± 14 (S.E.M.) fmol of [^{3}H]dihydroergocryptine per mg of membrane protein at saturation and demonstrated a dissociation constant of 8.6 ± 0.5 (S.E.M.) nM. These values were not significantly different ($P > 0.1$ in each case) from those obtained in renal membranes from untreated animals [192 ± 9 (S.E.M. fmol/mg of membrane protein and 13 ± 1 (S.E.M.) nM, respectively].

Specificity of catecholaminergic modulation of vasoconstriction in the isolated perfused kidney. Adrenergic agonists elicited vasoconstriction in the isolated perfused rat kidney (see table 1) with a potency order similar to that determined in the competition studies with [^{3}H]dihydroergocryptine and [^{3}H]prazosin. However, unlike the results obtained in the [^{3}H]dihydroergocryptine binding studies, (−)-epinephrine, (−)-norepinephrine and (−)-phenylephrine were more potent than clonidine. The vasoconstrictor responses elicited by the adrenergic agents also showed the expected stereoselectivity as the (−)-isomers of epinephrine and norepinephrine were 65- and 45-fold more potent than their respective (+)-isomers (see table 1). The specificity of the *alpha* receptor-mediated vasoconstrictor response was also examined by determining the ability of the antagonists to inhibit *alpha* agonist-induced vasoconstriction in the isolated perfused kidney (see table 1). In these studies, the *alpha* adrenergic agents were much more potent than the *beta* adrenergic antagnoist, (±)-propranolol. Moreover, in agreement with the [^{3}H]prazosin binding studies, but in contrast to [^{3}H]dihydroergocryptine and the [^{3}H]yohimbine binding studies, the following order of potency was observed: prazosin > phentolamine ≫ yohimbine.

Correlation of "*in vitro*" and binding studies. Figure 6 shows correlations of the relative potencies of adrenergic agonists and antagonists in the isolated-perfused kidney studies and the ability of these same compounds to displace [^{3}H]dihydroergocryptine and [^{3}H]prazosin from membrane-binding sites. Although close correlations were observed with both agents, the agreement between the *in vitro* and binding data was closer with [^{3}H]prazosin ($r = 0.93$) than for [^{3}H]dihydroergocryptine ($r = 0.88$). In addition, as determined by analysis of covariance (Zar, 1974), the slope (0.9) of the regression line relating the functional and [^{3}H]prazosin data was not significantly different from unity (the slope of the line of identity). In contrast, the slope of the regression line observed with [^{3}H] dihydroergocryptine (0.6) differed significantly from unity ($P < .01$) (see fig. 6).

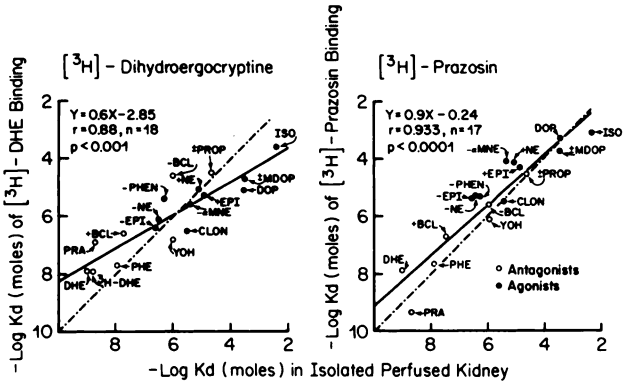

Fig. 6. Correlations between the apparent dissociation constants (K_D) determined in competitive inhibition studies with [^{3}H]dihydroergocryptine and [^{3}H]prazosin and those determined in the isolated perfused kidney (see text for details). The unbroken lines are the linear regression lines relating the data and the broken lines represent the lines of identity between the two systems. Abbreviations: DHE, dihydroergocryptine; PHE, phentolamine; PRA, prazosin; BCL, butaclamol; YOH, yohimbine; EPI, epinephrine; CLON, clonidine; NE, norepinephrine; PHEN, phenylephrine; αMNE, α-methylnorepinephrine; DOP, dopamine; PROP, propranolol; MDOP, methyldopamine; and ISO, isoproterenol.

Discussion

In the present study, binding sites with *alpha* adrenergic specificity have been identified in rat renal membranes by using nonselective ([^{3}H]dihydroergocryptine), *alpha*-1 selective ([^{3}H]prazosin) and *alpha*-2 selective ([^{3}H]yohimbine) antagonists. Binding with each of the radioligands was rapid, saturable and reversible (see figs. 1–5) and Scatchard plots of equilibrium binding data were linear for all three radioligands. With [^{3}H] dihydroergocryptine, the linearity of the Scatchard plot is probably due to the fact that this radioligand binds with similar affinity to both *alpha*-1 and *alpha*-2 receptors, rather than to a single class of homogeneous receptor sites. Thus, consistent with previous reports (Hoffman, *et al.*, 1979), the K_D values of unlabeled dihydroergocryptine were not markedly different, whether determined in competitive inhibition studies with [^{3}H]prazosin or [^{3}H]yohimbine. Furthermore, "pseudo" Hill coefficients calculated from inhibition studies with [^{3}H]dihydroergocryptine were less than one for unlabeled prazosin (0.7) and yohimbine (0.7), but close to unity for unlabeled dihydroergocryptine (1.02) and phentolamine (0.91). This finding and the observation that the concentration of receptor sites determined with [^{3}H]dihydroergocryptine (212 fmol/mg of protein) was approximately equal to the sum of the binding site concentrations obtained with the *alpha*-1 and *alpha*-2 selective radioligands (57 and 170 fmol/mg of protein, respectively) additionally suggest that [^{3}H]dihydroergocryptine labels both *alpha*-1 and *alpha*-2 adrenergic receptors.

In contrast, the linearity of the Scatchard plots obtained from the [^{3}H]prazosin and [^{3}H]yohimbine equilibrium binding studies suggests that these agents bind to a single class of receptor sites. The Hill coefficients (n_H) obtained from the equilibrium binding data with these selective radioligands were not significantly different from unity (n_H = 1.00, [^{3}H]prazosin; n_H = 0.97, [^{3}H]yohimbine). Moreover, it is likely that the binding of [^{3}H]prazosin to *alpha*-2 receptors or [^{3}H]yohimbine to *alpha*-1 receptors would have been negligible; as competitive inhibition studies with the unlabeled selective antagonists indicated that at the maximum concentrations of [^{3}H]prazosin and [^{3}H]yohimbine used in the equilibrium binding studies, less than 5% of *alpha*-2 or *alpha*-1 receptors, respectively, would have been labeled by the tritiated compounds.

Alpha adrenergic specificity of radioligand binding to the rat renal membranes was observed with each of the tritiated antagonists, as *alpha* adrenergic agents competed more potently for binding than did dopaminergic, *beta* adrenergic or serotonergic agents (see table 1). Stereospecificity of binding was also evidenced by the finding that (+)-isomers were less potent than (−)-isomers, whereas other vasoactive agents did not compete for [^{3}H]dihydroergocryptine binding even when tested in concentrations up to 10^{-3} M. The relative order of potency of adrenergic agents, however, differed for each of the three radioligands in a manner consistent with their selectivity or lack of selectivity for *alpha*-1 or *alpha*-2 receptors. The *alpha*-1 selective agonists (−)-norepinephrine and (−)-phenylephrine, for example, had a greater affinity at *alpha*-1 receptors (determined in competitive inhibition studies with [^{3}H]prazosin) than the *alpha*-2 selective agonist, (−)-α-methylnorepinephrine. Similarly, the affinity of antagonists at *alpha*-1 receptors declined in the order: prazosin > phentolamine > yohimbine. Yohimbine had a greater affinity at *alpha*-2 receptors than phentolamine or prazosin, whereas the affinities of *alpha*-1 and *alpha*-2 selective agonists and antagonists were less discrepant when determined by competitive inhibition studies with [^{3}H] dihydroergocryptine (see table 1). Classification of *alpha* receptors into *alpha*-1 and *alpha*-2 subtypes is more problematic with agonists than with antagonists (Hoffman *et al.*, 1979). For example, some agonists such as the *alpha*-2 selective agent, clonidine, have a higher affinity at *alpha*-1 receptors than the *alpha*-1 selective agonist, (−)-phenylephrine (Starke and Docherty, 1980). In functional studies, however, (−)-phenylephrine is more potent than clonidine (Starke and Docherty, 1980). For these reasons, and as the ultimate goal of binding studies is to gain insight into the molecular mechanisms by which adrenergic agents elicit physiological responses, we attempted to confirm the results obtained in the binding studies by examining an appropriate and quantifiable functional response (*i.e.*, renal vasoconstriction).

As shown in figure 6, the [^{3}H]dihydroergocryptine binding data correlated with that determined in the isolated perfused kidney. In the functional studies, however, renal vasoconstriction was clearly an *alpha*-1 receptor-mediated response as the *alpha*-1 selective agonists [(−)-norepinephrine and (−)-phenylephrine] and antagonist (prazosin) were more potent than the *alpha*-2 selective agonists [(−)-α-methylnorepinephrine and clonidine] and antagonist (yohimbine), respectively. With [^{3}H]dihydroergocryptine, the K_D values of *alpha*-1 and *alpha*-2 selective agents were not markedly different, suggesting (as discussed above) that a component of the binding with this nonselective *alpha* radioligand was to *alpha*-2 receptors. It is thus of interest that despite the reasonable correlation between the functional and [^{3}H]dihydroergocryptine binding data, the slope of the regression line (0.6) relating these data was significantly less than unity (the slope of the line of identity between the two systems).

In contrast to the results obtained with [^{3}H]dihydroergocryptine, there was closer agreement between the functional and [^{3}H]prazosin binding studies. Thus, there was a high degree of correlation (r = 0.93) between the K_D values observed in the functional and [^{3}H]prazosin studies, and the slope of the regres-

sion line (0.9) relating these data was not significantly different from that of the line of identity. In addition, the affinity of antagonists in both these systems declined in the order: prazosin > phentolamine ≫ yohimbine. The effects of agonists were also in agreement with those predicted for an *alpha*-1 coupled event (Starke and Docherty, 1980). For example, the *alpha*-1 selective agonist, (−)-norepinephrine, had both greater affinity and potency than the *alpha*-2 selective agent, (−)-α-methylnorepinephrine (table 1), whereas the *alpha*-2 selective agonist, clonidine, had higher affinity in the binding studies but a lower potency in the isolated perfused binding studies than the *alpha*-1 selective agent, (−)-phenylephrine (table 1). Only selected agents were examined in competitive inhibition studies with [^{3}H]yohimbine; however, as indicated above, the K_D values obtained for these agents clearly suggest that in contrast to the *alpha* receptor mediating renal vasoconstriction, that labeled by [^{3}H]yohimbine is of the *alpha*-2 subtype.

In agreement with previous findings (U'Pritchard and Snyder, 1979), sympathetic denervation with 6-hydroxydopamine did not alter the total renal *alpha* receptor concentration. This may be due to either of several possibilities. One is that while denervation may destroy prejunctional *alpha* receptors, the reduction in binding to prejunctional receptors may be masked by an increase in postjunctional receptors resulting from the decrease in neurotransmitter release. The second possibility is that prejunctional *alpha*-2 receptors may constitute such a small fraction of the total *alpha* receptor pool that their loss is not detectable in the binding assay. In this regard, it should be noted that Robie (1980) found no evidence that prejunctional *alpha* receptors are functionally significant in the dog kidney. Thus, the third and most likely possibility is that both *alpha*-1 and *alpha*-2 receptors identified in the binding studies are located postjunctionally. Whereas *alpha*-1 receptors appear to mediate renal vasoconstriction, the *alpha*-2 receptor coupled response remains to be defined. On the basis of pharmacological studies, Drew and Whiting (1979) have been unable to identify renal postjunctional vascular *alpha*-2 receptors. In contrast, Young and Kuchar (1980) have recently reported that on the basis of an autoradiographic technique using [^{3}H]clonidine, *alpha*-2 receptors appear to be located predominantly on proximal tubules. As an *alpha* adrenergic mechanism appears to be involved in proximal tubular sodium reabsorption (Slick *et al.*, 1975; Benesath *et al.*, 1972; Gottschalk, 1979), it is possible that renal *alpha*-2 receptors may mediate this effect.

The identification of rat renal-membrane binding sites with *alpha* adrenergic specificity has previously been reported using [^{3}H]clonidine and the *alpha*-1 selective antagonist, WB 4101 (U'Pritchard and Snyder, 1979). In these studies, *alpha*-2 receptors accounted for only 25% of the total renal *alpha* receptor population. This differs from the 65% that we found using [^{3}H]yohimbine. As no functional correlates were obtained in this study (U'Pritchard and Snyder, 1979), it is possible that this difference, as discussed previously, is due to the lesser utility of clonidine to discriminate between *alpha*-1 and *alpha*-2 receptors on the basis of binding studies. An alternative explanation is the fact that U'Pritchard and Snyder used a "crude" membrane preparation in their studies. This preparation results in greater nonspecific binding of the radioligands; however, it is less likely to be associated with loss of *alpha* receptors during the preparative procedures, which may account for the lesser proportion of *alpha*-1 receptor observed in our studies. To investigate this possibility, additional binding studies were performed using the same membrane preparation described by U'Pritchard and Snyder (1979). In these studies, *alpha*-1 and *alpha*-2 receptor concentrations were similar to those observed in the more purified membrane preparations (data not shown) and the ratio of *alpha*-1 to *alpha*-2 receptors remained unchanged.

In conclusion, binding sites with *alpha* adrenergic specificity have been identified in rat renal membranes. Both *alpha*-1 and *alpha*-2 receptors appear to be present in a ratio of 1:3. [^{3}H] Prazosin binds selectively to *alpha*-1 adrenergic receptors and can thus be used to quantitatively assess this receptor subtype as it relates to renal vasoconstriction. [^{3}H]Dihydroergocryptine binds to both *alpha*-1 and *alpha*-2 receptors and can be used to estimate the total renal *alpha* receptor population. [^{3}H] Yohimbine binds to renal *alpha*-2 receptors, the functions of which remain to be characterized. These receptors might be involved in controlling renin release (Pettinger *et al.*, 1976; Berthelsen and Pettinger, 1977) or regulating the tubular handling of sodium (Slick *et al.*, 1975; Benesath *et al.*, 1972; Gottschalk, 1979; Young and Kuhar, 1980).

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