MOLECULAR PHARMACOLOGY, 22:589-594

Characterization of Adenylate Cyclase-Coupled *Alpha*$_2$-Adrenergic Receptors in Rat Renal Cortex Using [^{3}H]Yohimbine

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Received March 22, 1982; Accepted June 15, 1982

SUMMARY

Alpha$_2$ adrenergic receptors in rat renal cortex were measured with the antagonist ligand [^{3}H]yohimbine. Renal cortical membranes contained 120 ± 11.7 (SE) fmoles of binding sites per milligram of protein (n = 9). Their affinity for [^{3}H]yohimbine was 10.4 ± 0.5 (SE) nM (n = 9) from equilibrium studies and 7.2 ± 3.3 (n = 4) from kinetic measurements. *Alpha*-adrenergic agonists and antagonists bound [^{3}H]yohimbine sites with affinities consistent with *alpha*$_2$-receptor binding. The slope factors for the binding of all antagonists tested were close to 1, whereas agonists bound with slope factors of less than 1, consistent with the presence of receptors of more than one affinity. The *alpha*$_2$-receptors measured with [^{3}H]yohimbine appeared to be coupled to the inhibition of adenylate cyclase. The affinities of *alpha*-adrenergic catecholamines measured in cyclase inhibition studies were similar to their affinities in binding studies when these were performed under conditions optimal for adenylate cyclase inhibition. Both sodium ion and GTP are required for maximal inhibition of renal cortical adenylate cyclase [Woodcock, E. A., C. I. Johnston, and C. A. Olsson. *J. Cyclic Nucleotide Res.* 6:261-271, 1980)]. Each of these factors produced a 10- to 20-fold decrease in the binding affinity of epinephrine. Together, a 100- to 200-fold decrease was produced. The concentrations of both sodium ion and GTP required to decrease the affinity of epinephrine were similar to the concentrations required for adenylate cyclase inhibition. This suggested that similar mechanisms were involved in decreasing the affinity of agonists for the *alpha*$_2$-receptor and promoting agonist-induced inhibition of adenylate cyclase.

INTRODUCTION

The initial classification of *alpha*-adrenergic receptors was based on functional and anatomical criteria differentiated into "presynaptic" and "postsynaptic" categories (1). The presynaptic *alpha*-receptors were thought to be located on sympathetic nerve terminals where they mediated feedback inhibition of norepinephrine release. They were selectively sensitive to yohimbine, clonidine, and *alpha*-methylnorepinephrine, unlike the postsynaptic receptors, which were sensitive to prazosin, WB-4101, phenylephrine, and methoxamine. More recent studies have shown that *alpha*-receptors with a pharmacological specificity similar to that of presynaptic receptors are found also in non-neural tissues such as platelets (2) and adipocytes (3). Moreover, direct binding studies have failed to demonstrate decreased *alpha*$_2$-receptor concentrations following denervation, indicating that the majority of these were postsynaptic (4). For these reasons, classification of *alpha*-receptors into *alpha*$_1$ and *alpha*$_2$ categories on the basis of relative drug potencies seems more universally applicable (5).

Alpha$_2$-receptors have been shown to be coupled to adenylate cyclase inhibition in platelets (2), adipocytes (3), neuroblastoma × glia hybrid cells (6), and liver (7). In each of these tissues, inhibition required GTP and was enhanced by monovalent cations (8). In platelets (9) and liver (10), binding of agonist compounds to *alpha*$_2$-receptors is modulated by sodium ion and guanyl nucleotides. We have recently reported specific inhibition of adenylate cyclase mediated by *alpha*$_2$-receptors in rat renal cortex (11, 12). Maximal inhibition of the kidney enzyme also required sodium ion and GTP. To investigate further these renal cortical *alpha*$_2$-receptors and their relationship to adenylate cyclase we have used and examined in detail the effects of sodium ion and GTP on the binding properties of the receptors.

METHODS

Materials. [*methyl*-^{3}H]Yohimbine, specific activity 75-90 Ci/mmole, was obtained from Searle Nucleonics. [α-^{32}P]ATP (triethylammonium salt), specific activity 350 Ci/mmole, and [2-^{3}H]adenosine-3',5'-cyclic monophosphate (ammonium salt), specific activity 30-50 Ci/mmole, were obtained from the Radiochemical Centre (Amersham, England).

The following compounds were obtained from Sigma Chemical Company (St. Louis, Mo.): yohimbine hydrochloride, Tris, (−)-epinephrine bitartrate, (−)-arterenol

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bitartrate, (−)-isoproterenol HCl, GTP, Gpp(NH)p,[1] ATP, cyclic AMP, caffeine, bovine serum albumin, pargyline, phosphoryl creatine (Tris salt), creatine kinase, neutral alumina, Dowex 50-W4-400 ion exchange resin, ascorbic acid, adenosine deaminase, and triethanolamine. (+)-Epinephrine bitartrate was obtained from KEK Fine Chemicals. Propranolol was supplied by ICI, prazosin by Pfizer, α-methylnorepinephrine and phenylephrine by Sterling Pharmaceuticals, and methoxamine by Wellcome.

Preparation of renal cortical membranes. Adult Sprague-Dawley rats (150–250 g) were killed by cervical dislocation. Kidneys were back-perfused with saline through the renal vein, removed, and hemisected; cortices and medullae were separated. Cortices were homogenized with a Potter-Elvejhem homogenizer in buffer containing 50 mM Tris-HCl (pH 7.7), 10 mM $MgSO_4$, 0.25 M sucrose, 10^{-4} M (−)-isoproterenol, and 10^{-4} M GMP [to remove bound GDP (13)] at 0°. This treatment did not alter [^{3}H]yohimbine binding nor the adenylate cyclase assay. Agonist affinity was slightly increased (1.5- to 2-fold) by the treatment. Homogenates were diluted to approximately 100 ml/10 kidneys and centrifuged twice at 3000 × *g* for 5 min at 5°. The final supernatant was centrifuged at 30,000 × *g* for 15 min. The pellet was washed once with the above sucrose buffer and then three times with buffer without isoproterenol and GMP. The final pellet was resuspended in 50 mM Tris (pH 7.7)/10 mM $MgSO_4$/1 mM mercaptoethanol at a protein concentration of 5–10 mg/ml. Protein concentration was measured by the method of Lowry *et al.* (14), using bovine serum albumin as standard. Membrane preparations were used immediately.

[^{3}H]Yohimbine binding. Incubation media, in a final volume of 150 μl, contained [^{3}H]yohimbine (1–50 nM), 50 mM Tris-HCl (pH 7.7), 10 mM $MgSO_4$, 1 mM mercaptoethanol (as a reducing agent for the catecholamines), renal membranes (1–3 mg of protein per milliliter), and other additions as indicated. Incubation was carried out at 25° for 20 min and was terminated by dilution with 2.5 ml of 50 mM Tris-HCl (pH 7.7)/10 mM $MgSO_4$ at 0° followed immediately by filtration through Whatman GF/C glass-fiber filters. The filters were washed with an additional 7.5 ml of ice-cold buffer.

Nonspecific binding was defined as binding not displaceable by 10^{-4} M (−)-epinephrine and represented 30% of the total binding at 10 nM [^{3}H]yohimbine.

For competition experiments where the affinities of nonradioactive drugs were measured, the [^{3}H]yohimbine concentration was 5 nM. EC_{50} values and slope factors were calculated by logit analysis. Where the slope factors equaled 1, K_I values were calculated by using the formula $K_I = EC_{50}/(1 + [L]/K_L)$ (15).

Adenylate cyclase assay. Adenylate cyclase assays, in a final volume of 0.1 ml at pH 7.6, contained 50 mM triethanolamine HCl, 10 mM $MgSO_4$, 5 mM KCl, 0.1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid, 10 mM caffeine, 20 mM phosphorylcreatine, creatine kinase (10 μg/ml), 0.1 mM cyclic AMP, bovine serum albumin (1 mg/ml), 10 μM pargyline, adenosine deaminase (10 μg/ml) (to prevent generation of adenosine from ATP), 0.05% ascorbic acid, 0.1 mM [α-^{32}P]ATP (100 cpm/pmole), kidney homogenate (0.3–0.4 mg of protein), and other additions as indicated on the figures.

Incubation was carried out at 25° for 10 min and was terminated by adding 0.2 ml of 1 M perchloric acid. Approximately 10,000 cpm of [^{3}H]cyclic AMP were added to each tube as a recovery marker. Following centrifugation to remove precipitated protein, cyclic AMP was isolated using sequential chromatography over Dowex and neutral alumina columns (16). Recovery of cyclic AMP was 60–70%.

Under the conditions used, cyclic AMP generation was linear both with time and with protein concentration. No ^{32}P-labeled product other than cyclic AMP could be detected by thin-layer chromatography. Duplicate samples agreed within 3%.

RESULTS

Saturability, affinity, and kinetics of [^{3}H]yohimbine binding. Binding of [^{3}H]yohimbine to renal cortical membranes was saturable, and Scatchard analysis of the binding data produced a straight line indicating a single class of noncooperative binding sites. The affinity of [^{3}H]yohimbine for the sites was 10.4 ± 0.5 (SE) nM (n = 9) and there were 120 ± 11.7 (SE) fmoles of binding sites per milligram of protein (Fig. 1).

Binding was rapid and rapidly reversible. Binding reached equilibrium in less than 15 min at all [^{3}H]yohimbine concentrations tested (Fig. 2). Under the conditions used, not more than 5% of the added ligand was bound. Therefore the binding reaction can be simplified to a pseudo-first order reaction, and the following equation to describe the approach to equilibrium applies:

$$\mathrm{Ln}\frac{[HR]_{eq}}{[HR]_{eq} - [HR]} = (k_{+1}\cdot[L] + k_{-1})t$$

where $[HR]$ is the concentration of receptor-ligand complex at time t; $[HR]_{eq}$ is the concentration at equilibrium; k_{+1} and k_{-1}, respectively, are the forward and reverse rate constants; and $[L]$ is the radioligand concentration. The kinetic data were plotted according to this equation, producing linear plots of $\log([HR]_{eq}/[HR]_{eq}-[HR])$

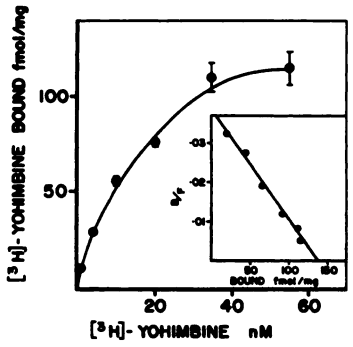

FIG. 1. *[^{3}H]Yohimbine binding to renal cortical membranes: affinity and saturability of binding*

The saturation binding curve shows the results of nine experiments with results expressed as means ± standard errors. The *inset* shows a representative Scatchard plot from a single experiment.

[1] The abbreviation used is: Gpp(NH)p, guanylyl 5′-imidodiphosphate.

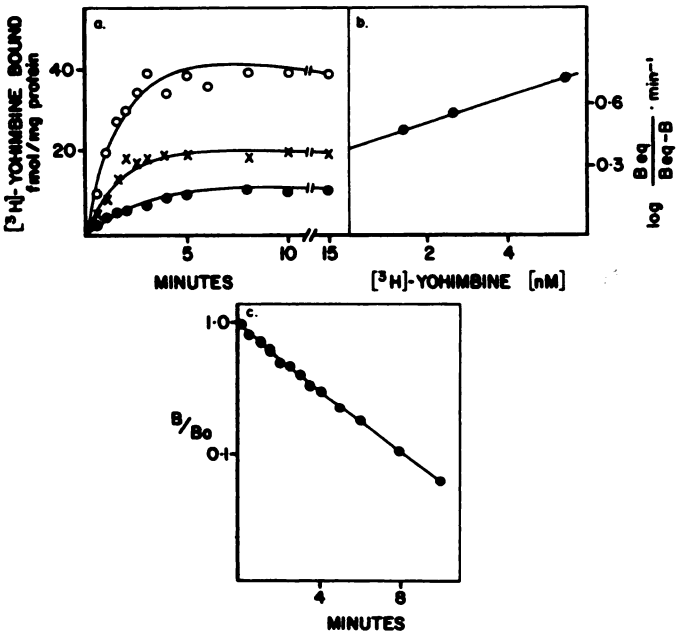

FIG. 2. *Kinetics of [^{3}H]yohimbine binding to renal cortical membranes*

a. Rate of binding of [^{3}H]yohimbine: O——O, 1.5 nM [^{3}H]yohimbine; X——X, 2.6 nM [^{3}H]yohimbine; ●——●, 5.5 nM [^{3}H]yohimbine.

b. Values for log $B_{eq}/(B_{eq} - B)$ were plotted against time, and the slopes of the resulting lines were plotted against the [^{3}H]yohimbine concentration. The slope of this line equals k_{+1} and the intercept equals k_{-1}. B_{eq} is the amount (femtomoles) of [^{3}H]yohimbine bound at equilibrium. B is the amount (femtomoles) of [^{3}H]yohimbine bound at time t.

c. Dissociation of bound [^{3}H]yohimbine. Renal membranes were incubated with 5 nM [^{3}H]yohimbine for 20 min. Dissociation was initiated by adding 10^{-4} M (−)-epinephrine. Shown is a representative experiment. The experiment was performed four times.

against incubation time. The slope of the line so obtained was plotted against the [^{3}H]yohimbine concentration. The slope of this line equals k_{+1} and the intercept on the ordinate equals k_{-1} (Fig. 2a and b). Average values for k_{+1} were 0.031 ± 0.006 (SE) nM^{-1} min^{-1} and for k_{-1} 0.22 ± 0.05 (SE) min^{-1} (n = 4). K_d values, calculated as k_{-1}/k_{+1}, averaged 7.2 ± 3.3 (SE) nM (n = 4). This value is in good agreement with values obtained by equilibrium methods.

Direct dissociation experiments were carried out to obtain an alternative value of k_{-1}. Membranes were incubated for 20 min at 25° with 5 nM [^{3}H]yohimbine. Dissociation was initiated by adding 10^{-4} M (−)-epinephrine. Dissociation of specifically bound [^{3}H]yohimbine was monoexponential, and the k_{-1} value calculated from four experiments was 0.28 ± 0.03 (SE) min^{-1} (Fig. 2c).

Specificity of [^{3}H]yohimbine binding sites. The affinities of a number of adrenergic agonists and antagonists were measured in competition experiments with [^{3}H] yohimbine. Results are shown in Table 1. Nonradioactive yohimbine was the most potent competitor, followed by the nonselective *alpha*-antagonist phentolamine. Both of these agents were more potent competitors than were the *alpha*$_1$-selective antagonist prazosin. All three antagonists produced competition curves with slope factors close to 1, indicating a single class of binding sites for all of these compounds. Of the catecholamines, the *alpha*$_2$-selective agonist α-methylnorepinephrine and (−)-epinephrine had similar affinities and were most potent followed by the (−)-isomer of norepinephrine. (+)-Epinephrine was more than 10 times weaker than the (−)-isomer demonstrating stereospecificity of binding. The *alpha*$_1$-selective agonist compounds phenylephrine and methoxamine were weak competitors for [^{3}H]yohimbine binding. Agonist competition curves were more shallow than those of antagonists, as reflected by slope factors of less than 1, indicating the presence of more than one affinity binding site for all agonists tested. Clonidine competition curves had slope factors of 1, indicating that clonidine acts largely as an antagonist at these renal *alpha*$_2$-receptors. Clonidine was previously shown to have only partial agonist activity in inhibiting renal cortical adenylate cyclase (11).

Relationship of [^{3}H]yohimbine binding sites to alpha-adrenergic inhibition of adenylate cyclase. As we have demonstrated previously, the addition of the *alpha*$_2$-adrenergic agonists epinephrine, norepinephrine, and *al-*

TABLE 1

Specificity of [^{3}H]yohimbine binding sites in renal cortical membranes

The [^{3}H]yohimbine concentration was 5 × 10^{-9} M. Values shown are means ± standard error of the mean. All experiments were performed at least three times.

Drug	EC_{50}	Slope factor	Na + GTP[a]	
			EC_{50}	Slope factor
	M		M	
Yohimbine	12.1 ± 0.2 × 10^{-9}	0.98 ± 0.05		
Phentolamine	1.6 ± 0.1 × 10^{-8}	1.00 ± 0.05		
Prazosin	1.7 ± 0.3 × 10^{-7}	0.99 ± 0.06		
Phenylephrine	5.2 ± 0.8 × 10^{-6}	0.76 ± 0.01		
Methoxamine	1.9 ± 0.4 × 10^{-5}	0.80 ± 0.09		
(−)-Isoproterenol	1.5 ± 0.4 × 10^{-4}	0.76 ± 0.02		
Clonidine	5.8 ± 0.3 × 10^{-8}	1.06 ± 0.06	2.1 ± 0.5 × 10^{-7}	1.04 ± 0.07
(−)-Epinephrine	9.7 ± 0.5 × 10^{-8}	0.63 ± 0.03	1.1 ± 0.1 × 10^{-5}	0.98 ± 0.05
(+)-Epinephrine	4.0 ± 0.7 × 10^{-6}	0.65 ± 0.05	3.9 ± 0.7 × 10^{-5}	0.98 ± 0.06
(−)-Norepinephrine	4.6 ± 0.2 × 10^{-7}	0.66 ± 0.04	3.6 ± 1.1 × 10^{-6}	1.01 ± 0.05
α-Methylnorepinephrine	1.3 ± 0.4 × 10^{-7}	0.61 ± 0.04	2.3 ± 0.9 × 10^{-6}	0.95 ± 0.03
Dopamine	6.7 ± 0.7 × 10^{-6}	0.68 ± 0.02	4.9 ± 1.4 × 10^{-5}	0.90 ± 0.04

[a] Plus 20 mM phosphorylcreatine and creatine kinase (10 μg/ml).

pha-methylnorepinephrine to renal cortical membranes produced concentration-dependent inhibition of adenylate cyclase activity (11) (Fig. 3). To investigate the relationship between [^{3}H]yohimbine binding sites and *alpha*$_2$-receptors coupled to adenylate cyclase, inhibition affinities of agonists measured in the two systems were compared. Affinities (K_I values) of agonists for adenylate cyclase inhibition were calculated as the concentration producing 50% of the maximal inhibition. Agonist binding experiments were carried out in the presence of 150 mM NaCl, 10^{-5} M GTP, 20 mM phosphorylcreatine, and creatine kinase (10 μg/ml) to simulate the conditions of the adenylate cyclase assay. Components of the adenylate cyclase assay other than NaCl and GTP had no effect on agonist binding. Binding affinities of agonists were reduced in the presence of these factors (Table 1). K_I values obtained in the two systems were similar, and the correlation coefficient calculated by the least-squares method was 0.98 (Fig. 3). As the maximal inhibition produced by epinephrine was only 20 ± 2% (SE), it was not possible to obtain K_I values for antagonists or partial agonists from adenylate cyclase inhibition studies. Neither NaCl nor GTP separately or in combination had any effect on the binding affinity of [^{3}H]yohimbine or the other antagonists tested, phentolamine and prazosin.

Effects of NaCl and GTP on binding of (−)-epinephrine. To investigate further the effects on agonist binding of factors required for adenylate cyclase inhibition, the affinity of epinephrine was investigated in the presence of either NaCl (150 mM) or GTP (10^{-4} M) (plus phosphorylcreatine and creatine kinase) or both NaCl and GTP together. Both NaCl and GTP decreased the average affinity of epinephrine. KCl at 150 mM did not decrease the affinity of epinephrine. In the presence of both factors the average affinity was further reduced (Fig. 4). The effects of the two factors were additive. The nonhydrolyzable GTP analogue GPP(NH)p (10^{-4} M) produced changes in affinity similar to those produced by GTP plus regenerating system (Table 2). Smaller shifts were observed in the absence of the GTP-regenerating system, indicating the presence of GTPase activity in the cortical membrane preparation.

Neither NaCl nor GTP (plus regenerating system) alone caused a significant change in the slope factor. However, in the presence of both NaCl and GTP together, the slope factor increased to 0.98 ± 0.05 (SE), indicating a single-affinity class of receptors for epinephrine under conditions optimal for adenylate cyclase inhibition (Table 2).

Correlation of agonist binding and adenylate cyclase inhibition. Sodium ion and GTP are required both for maximal inhibition of adenylate cyclase by *alpha*-adrenergic agonists and for maximal decrease in agonist affinity. Concentration dependences of the two effects on sodium ion and GTP were compared. Increasing concentrations of NaCl produced a progressive decrease in the average affinity of (−)-epinephrine. The same concentration range progressively enhanced adenylate cyclase inhibition by (−)-epinephrine. These experiments were performed in the presence of 10^{-4} M GTP (plus regener-

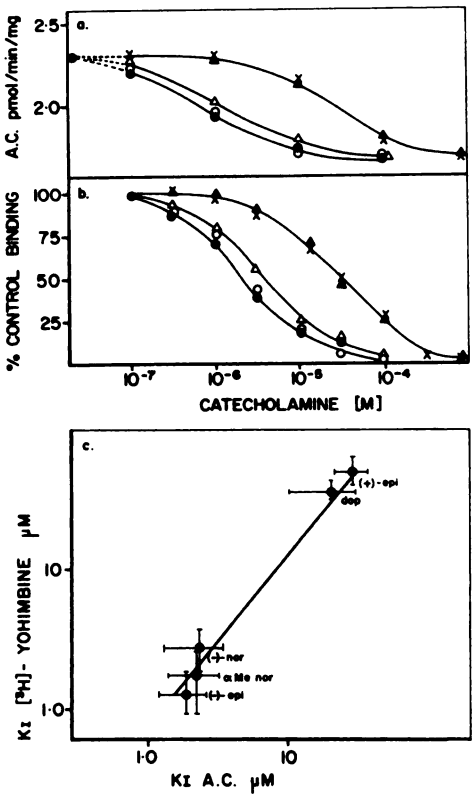

FIG. 3. *Correlation of alpha-adrenergic agonist affinities measured in competition binding experiments with their affinities for inhibition of adenylate cyclase*

Binding experiments and adenylate cyclase assays were carried out in the presence of 150 mM NaCl, 10^{-5} M GTP, 20 mM phosphorylcreatine, and creatine kinase (10 μg/ml).

a. Inhibition of adenylate cyclase by *alpha*-adrenergic agonists. b. Binding of *alpha*-adrenergic agonists. ●——●, (−)-Epinephrine; ○——○, α-methylnorepinephrine; ▲——▲, (−)-norepinephrine; △——△, (+)-epinephrine; X——X, dopamine.

c. Correlation of EC_{50} values obtained from adenylate cyclase inhibition and [^{3}H]yohimbine binding. *(+)-epi*, (+)-Epinephrine; *dop*, dopamine; *(−)-nor*, (−)-norepinephrine; *α Me nor*, α-methylnorepinephrine; *(−)-epi*, (−)-epinephrine.

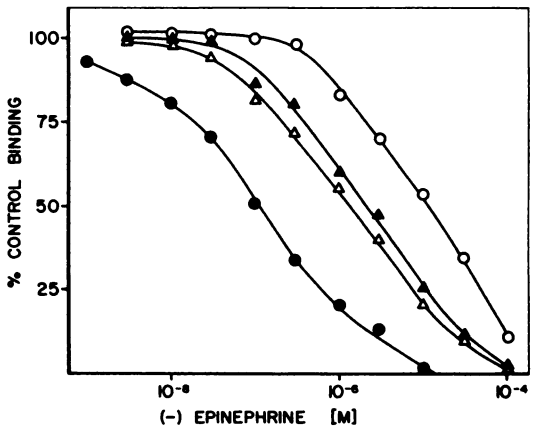

FIG. 4. *Modulation of epinephrine affinity for renal alpha*$_2$*-receptors by NaCl and GTP*

●——●, No additions; △——△, 10^{-5} M GTP plus 20 mM phosphorylcreatine and creatine kinase (10 μg/ml); ▲——▲, 150 mM NaCl; ○——○, NaCl plus GTP plus 20 mM phosphorylcreatine and creatine kinase (10 μg/ml).

TABLE 2

Modulation of (−)-epinephrine affinity by NaCl and GTP

The [^{3}H]yohimbine concentration was 5×10^{-9} M. EC_{50} values in the presence of NaCl, GTP, or Gpp(NH)p were significantly different from control ($p < 0.001$). EC_{50} values in the presence of both NaCl and GTP or NaCl and Gpp(NH)p were significantly different from values in presence of NaCl, GTP, or Gpp(NH)p alone ($p < 0.001$). Statistical significance was evaluated using Student's paired *t*-test.

	No addition	150 mM NaCl	10^{-5} M GTP[a]	NaCl + GTP[a]	10^{-5} M Gpp(NH)p	NaCl + Gpp(NH)p
EC_{50}	9.7 ± 0.5	2.6 ± 0.25	1.2 ± 0.01	1.1 ± 0.1	1.2 ± 0.08	9.4 ± 0.8
Concentration (M)	$\times 10^{-8}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$
Slope factor	0.63 ± 0.03	0.67 ± 0.03	0.65± 0.01	0.98 ± 0.05[b]	0.65 ± 0.01	0.98 ± 0.01[b]

[a] Plus 20 mM phosphorylcreatine and creatine kinase (10 μg/ml).

[b] $p < 0.001$ compared with control.

ating system). The relationship between adenylate cyclase inhibition and epinephrine affinity at four different NaCl concentrations is shown in Fig. 5. Results shown (Fig. 5d) are percentages of the maximal effect, which was measured using 300 mM NaCl. The correlation coefficient measured, using the least-squares method, was 0.95.

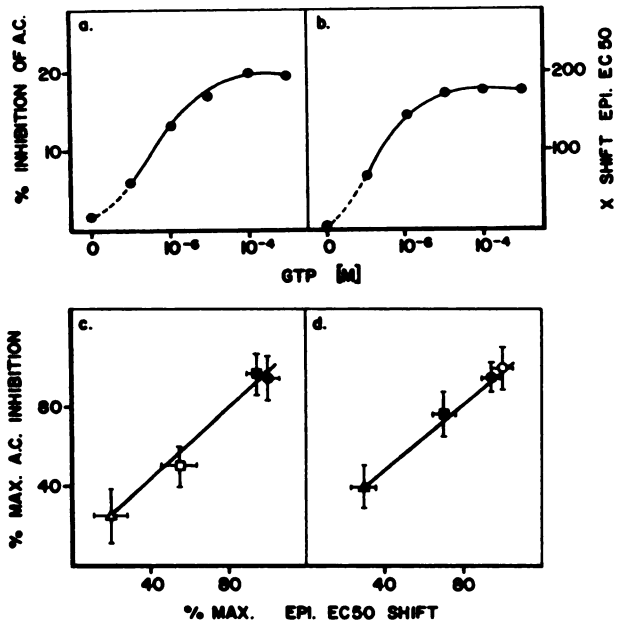

FIG. 5. *Relationship between adenylate cyclase inhibition and epinephrine affinity changes*

a and b. Effect of GTP on inhibition of adenylate cyclase by (−)-epinephrine (a) and the affinity of (−)-epinephrine for [^{3}H]yohimbine binding sites (b). Shown is a typical experiment. Average data from five experiments are shown in c.

c. The GTP concentration dependence of adenylate cyclase inhibition was compared with the concentration dependence for decreasing average (−)-epinephrine affinity. Results shown are percentages of the maximal effect, which was measured at 10^{-3} M GTP. The NaCl concentration was 150 mM, and all experiments were carried out in the presence of 20 mM phosphorylcreatine and creatine kinase (10 μg/ml). △, 10^{-7} M GTP; □, 10^{-6} M GTP; ■, 10^{-5} M GTP; ●, 10^{-4} M GTP.

d. The NaCl concentration dependence of adenylate cyclase inhibition was compared with the concentration dependence for decreasing average (−)-epinephrine affinity. Results shown are percentages of the maximal effect, which was measured at 300 mM NaCl. All experiments were carried out in the presence of 10^{-5} M GTP plus 20 mM phosphorylcreatine and creatine kinase (10 μg/ml). Experiments were performed in a manner similar to those shown in c. ▲, 50 mM NaCl; ■, 100 mM NaCl; ●, 150 mM NaCl; ○, 200 mM NaCl.

Similar experiments were performed at four different GTP concentrations (10^{-7}–10^{-4} M) in the presence of 0.15 M NaCl. Results are calculated as percentages of the maximal effect, which was measured at 10^{-3} M GTP. The same GTP-regenerating system was included in all experiments. The correlation coefficient was 0.93 (Fig. 5).

DISCUSSION

The recent documentation of *alpha*$_2$-receptors coupled to the inhibition of adenylate cyclase has added a new dimension to the modulation of cyclic AMP-dependent processes by adrenergic agonists. Thus cyclic AMP levels can be increased via *beta*-receptor stimulation or depressed by stimulation of *alpha*$_2$-receptors. We have previously demonstrated the existence of *alpha*$_2$-receptors inhibitory to adenylate cyclase in rat renal cortex. In this paper the properties of these receptors have been studied in detail using the antagonist ligand [^{3}H]yohimbine. Binding was rapid, reversible, and of high affinity. The *alpha*$_2$-selective drugs α-methylnorepinephrine and clonidine had high affinities for the sites, whereas the *alpha*$_1$-selective compounds prazosin, methoxamine, and phenylephrine had lower affinities. That these [^{3}H]yohimbine binding sites were *alpha*$_2$-receptors coupled to adenylate cyclase was strongly suggested by the correlation between the affinities of agonist compounds measured in adenylate cyclase inhibition experiments with those determined by competition for [^{3}H]yohimbine binding. Unfortunately, because the maximal inhibition observed was only 20%, this relationship could be investigated only for full agonists. Only catecholamines behaved as full agonists in inhibiting adenylate cyclase (11). The synthetic *alpha*$_2$-agonist clonidine had only partial agonist activity in inhibiting renal adenylate cyclase. Partial agonist activity of clonidine in inhibiting adenylate cyclase in platelets and adipocytes has also been reported (6, 17).

For all antagonist compounds tested—yohimbine, phentolamine, and prazosin—slope factors calculated from binding experiments were close to 1, indicating binding to a single class of sites. Agonists produced shallow competition curves with slope factors of less than 1, indicating that agonists bind to sites of more than one affinity. Under conditions optimal for adenylate cyclase inhibition (i.e., in the presence of NaCl and GTP), the slope factors of all agonists tested were close to 1. In kidney membranes, both NaCl and GTP were required to increase the slope factor of epinephrine binding to 1. Each of these factors alone caused a 10- to 20-fold de-

crease in the average affinity of epinephrine. The two factors appeared to act independently because, in combination, a 100- to 200-fold decrease in average affinity was observed. However, the two factors together were required to increase the slope factor to 1, implying some interaction between the two. Work with intact platelets has shown that extracellular sodium ion can modulate agonist affinity (18). However, the GTP binding site is thought to be located on the cytoplasmic side of the plasma membrane (19). Thus two different sites appear to be involved in modulation of affinity by sodium ion and guanyl nucleotides.

The data presented here for the kidney *alpha*$_2$-receptor are similar to those reported by others for *alpha*$_2$-receptors on platelets (20) and liver membranes (21). However, there are certain differences. In renal membranes, the slope factor of epinephrine binding increased to 1 only in the presence of both NaCl and GTP. The addition of either GTP or Gpp(NH)p alone did not significantly alter the slope factor. In a study of platelet membranes Michel *et al.* (20) reported a slope factor of 1 for epinephrine binding in the presence of 10^{-4} M Gpp(NH)p. In contrast, in liver the slope factor for epinephrine binding was not increased to 1 by either Gpp(NH)p or NaCl separately or in combination (21). These quantitative differences may be caused by technical difficulties in working with complex tissues or might reflect fundamental differences in the characteristics of *alpha*$_2$-receptors from the different tissue sources.

The decrease in affinity caused by sodium ion on platelet *alpha*$_2$-receptors was greater for full agonists than for partial agonists and was not observed with antagonists (22). We also found only a 3-fold shift in the affinity of clonidine in the presence of NaCl and Gpp(NH)p compared with the 100- to 200-fold shift observed with the catecholamines (Table 1). This suggests a relationship between agonist affinity changes and efficacy in inhibiting adenylate cyclase. Results reported here have demonstrated close similarity between concentrations of NaCl and GTP required to decrease epinephrine affinity and to maximize adenylate cyclase inhibition. These results strongly suggest that similar mechanisms are involved in the two processes and that the complex binding properties of agonists at renal *alpha*$_2$-receptors are a consequence of their coupling to the inhibition of adenylate cyclase.

REFERENCES

1. Langer, S. Z. Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmacol.* **60**:481–497 (1977).
2. Steer, M. L., and A. Wood. Regulation of human platelet adenylate cyclase by epinephrine, prostaglandin E, and guanine nucleotides: evidence for separate guanine nucleotide sites mediating stimulation and inhibition. *J. Biol. Chem.* **254**:10491–10797 (1979).
3. Aktories, K., G. Schultz, and K. H. Jakobs. Inhibition of hamster fat cell adenylate cyclase by prostaglandin E_1 and epinephrine: requirements for GTP and sodium ions. *F. E. B. S. Lett.* **107**:100–104 (1979).
4. U'Prichard, D. C., and S. H. Snyder. Distinct alpha-noradrenergic receptors differentiated by binding and physiological relationships. *Life Sci.* **24**:79–88 (1979).
5. Berthelson, S., and W. A. Pettinger. A functional basis for classification of α-adrenergic receptors. *Life Sci* **21**:595–606 (1977).
6. Sabol, S. L., and M. Nirenburg. Regulation of adenylate cyclase of neuroblastoma × glioma hybrid cells by α-adrenergic receptors: inhibition of adenylate cyclase mediated by α-receptors. *J. Biol. Chem.* **254**:1913–1920 (1979).
7. Jard, S., B. Cantau, and K. H. Jakobs. Angiotensin II and α-adrenergic agonists inhibit rat liver adenylate cyclase. *J. Biol. Chem.* **256**:2603–2606 (1981).
8. Jakobs, K. H. Inhibition of adenylate cyclase by hormones and neurotransmitters. *Mol. Cell. Cardiol.* **16**:147–156 (1979).
9. Michel, T., B. B. Hoffman, and R. J. Lefkowitz. Differential regulation of the α-2 adrenergic receptor by Na^+ and guanine nucleotides. *Nature (Lond.)* **288**:709–711 (1980).
10. Hoffman, B. B., D. F. Dukes, and R. J. Lefkowitz. Alpha-adrenergic receptors in liver membranes: delineation with subtype selective radioligands. *Life Sci.* **28**:265–272 (1981).
11. Woodcock, E. A., C. I. Johnston, and C. A. Olsson. Alpha-adrenergic inhibition of renal cortical adenylate cyclase. *J. Cyclic Nucleotide Res.* **6**:261–271 (1980).
12. Woodcock, E. A., and C. I. Johnston. Selective inhibition by epinephrine of parathyroid hormone stimulated adenylate cyclase in rat renal cortex. *Am. J. Physiol.* **242**:F721–F726.
13. Stadel, J. M., A. De Lean, and R. J. Lefkowitz. A high affinity β-adrenergic receptor complex is an intermediate for catecholamine stimulation of adenylate cyclase in turkey and frog erythrocyte membranes. *J. Biol. Chem.* **255**:1436–1441 (1980).
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
15. Chen, Y.-C., and W. H. Prusoff. Relationship between inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
16. White, A. A., and D. B. Karr. Improved two-step method for the assay of adenylate and guanylate cyclase. *Anal. Biochem.* **85**:451–460 (1978).
17. Schimmel, R. J., R. Serio, and K. K. McMahon. α-Adrenergic inhibition of cyclic AMP accumulation in hamster adipocytes. Similarity of receptor with α-2 receptors. *Biochim. Biophys. Acta* **632**:544–552 (1980).
18. Motulsky, H. J., S. J. Shattel, and P. A. Insel. Characterisation of α2-adrenergic receptors on human platelets using [^{3}H]yohimbine. *Biochem. Biophys. Res. Commun.* **97**:1562–1570 (1980).
19. Rodbell, M. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature (Lond.)* **284**:17–22 (1980).
20. Tsai, B. S., and R. J. Lefkowitz. Agonist-specific effects of guanine nucleotides on *alpha*-adrenergic receptors in human platelets. *Mol. Pharmacol.* **16**:61–68 (1979).
21. Hoffman, B. B., D. F. Dukes, and R. J. Lefkowitz. Alpha-adrenergic receptors in liver membranes: delineation with subtype selective radioligands. *Life Sci* **28**:265–273 (1981).
22. Tsai, B. S., and R. J. Lefkowitz. Agonist-specific effects of monovalent and divalent cations on adenylate cyclase-coupled *alpha* adrenergic receptors in rabbit platelets. *Mol. Pharmacol.* **14**:540–548 (1978).

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