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Occupancy of *Alpha*$_1$-Adrenergic Receptors and Contraction of Rat Vas Deferens

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SUMMARY

The interaction of agonists and antagonists with *alpha*$_1$-adrenergic receptors in rat vas deferens was examined using radioligand binding assays and contractility measurements. ^{125}I-Labeled BE 2254 (^{125}IBE) was found to bind rapidly and reversibly to a single class of high-affinity binding sites in homogenates of rat vas deferens. The k_1 for association was 3.8×10^7 1/mole-sec, the k_{-1} for dissociation was 2.3×10^{-3} sec^{-1}, and the K_D was 105 pM. The order of potency for antagonists inhibiting ^{125}IBE binding was prazosin > indoramin > phentolamine > yohimbine. Norepinephrine, phenylephrine, and other *alpha*-adrenergic agonists produced dose-dependent contractions of whole vas deferens *in vitro*. This contractile response was competitively inhibited by *alpha*-adrenergic blocking drugs with the same potency order observed for inhibition of specific ^{125}IBE binding. Comparison of pA$_2$ values for *alpha*$_1$- and *alpha*$_2$-selective antagonists competitively inhibiting contractile responses to norepinephrine, epinephrine, or phenylephrine suggested that these drugs caused their contractile effects solely through *alpha*$_1$-adrenergic receptors, and that there were no *alpha*$_2$-adrenergic receptors mediating contraction in this tissue. The pA$_2$ values for antagonist inhibition of *alpha*-adrenergic receptor-mediated contractile responses were highly correlated ($r = 0.995$) with the K_D values for antagonist inhibition of ^{125}IBE binding in this tissue. The EC$_{50}$ values for partial agonists were also highly correlated with the K_D values for inhibition of ^{125}IBE binding in vas deferens. However, the EC$_{50}$ values of full agonists in causing contraction were in general 10- to 100-fold lower than the K_D values for inhibiting ^{125}IBE binding, possibly representing a substantial "spare receptor" population in this tissue. The results suggest that rat vas deferens contains a homogeneous population of *alpha*$_1$-adrenergic receptors mediating the contractile response to norepinephrine, that these receptors can be directly labeled with ^{125}IBE, and that there may be a nonlinear relationship between agonist occupancy of *alpha*$_1$-adrenergic receptors and the functional response of this tissue.

INTRODUCTION

It has recently become clear that there are at least two discrete types of *alpha*-adrenergic receptor with different pharmacological properties (1, 2). *Alpha*$_1$-adrenergic receptors are generally thought to mediate vasoconstriction caused by norepinephrine released from sympathetic neurons and are selectively stimulated by phenylephrine and blocked by prazosin. *Alpha*$_2$-adrenergic receptors are thought to regulate norepinephrine release from presynaptic nerve terminals and are selectively stimulated by clonidine and blocked by yohimbine. Recent evidence has shown, however, that the distinction between these two receptors should be based solely on pharmacological criteria, and not on functional criteria, since *alpha*$_1$- and *alpha*$_2$-receptors may coexist in some smooth muscles and mediate the same functional response (2–4).

Radioligands for the direct study of *alpha*-adrenergic receptors have been available for several years (5, 6). However, since many of these compounds, such as [^{3}H]dihydroergocryptine and [^{3}H]WB 4101,[2] label both *alpha*$_1$- and *alpha*$_2$-receptors to varying extents (7, 8) and others, such as [^{3}H]clonidine and [^{3}H]*p*-aminoclonidine, are agonists with complicated binding characteristics (9), the results obtained with these compounds have sometimes been difficult to interpret (see ref. 10). Recently the selective antagonists [^{3}H]prazosin (11) and [^{3}H]yohimbine (12) have been reported to label *alpha*$_1$- and *alpha*$_2$-adrenergic receptor sites, respectively, in rat

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[2] The abbreviations used are: WB 4101, 2-[*N*-(2,6-dimethoxyphenoxyethyl)]-aminoethyl-1,4-benzodioxane; ^{125}IBE, ^{125}I-labeled BE 2254 (2-[β-(4-hydroxyphenyl)-ethylaminomethyl]-tetralone); KRB buffer, Krebs-Ringer bicarbonate buffer.

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brain. In addition, Engel and Hoyer (13) and Glossman *et al.* (14) have reported the development of an iodinated high-affinity *alpha*$_1$-adrenergic receptor antagonist, ^{125}IBE, which selectively labels binding sites with the characteristics of *alpha*$_1$-adrenergic receptors in rat brain. The high-affinity and specific activity of this iodinated compound makes it particularly suitable for use in tissues with a low receptor density or where the quantity of available tissue is small.

Although the binding sites labeled by many of these compounds have characteristics expected of *alpha*-adrenergic receptors, the pharmacological properties of these binding sites are not usually directly compared with the pharmacological characteristics of a receptor-mediated response in the same tissue. Direct binding studies usually utilize brain tissue because of its high receptor density, whereas receptor-mediated responses have classically been measured using various muscles *in vitro*. Although comparisons of the pharmacological characteristics of [^{3}H]prazosin and [^{3}H]WB 4101 binding sites in rat brain and *alpha*-receptor-mediated vasoconstriction have been made (15, 16), a direct comparison of receptor occupancy and tissue response would necessitate making both of these measurements on the same tissue.

Rat vas deferens is a smooth muscle preparation which is often used to study *alpha*-adrenergic receptor-mediated contractile responses (17, 18). In this paper, we report the use of ^{125}IBE to label *alpha*$_1$-adrenergic receptors in rat vas deferens and directly compare the binding characteristics of *alpha*$_1$-adrenergic receptors in this tissue with the pharmacological properties of the *alpha*-adrenergic receptor-mediated contractile response.

EXPERIMENTAL PROCEDURES

Materials. The drugs used were obtained from the following sources: phenylpropanolamine HCl, crystalline reserpine, norepinephrine bitartrate, epinephrine bitartrate, (−)-phenylephrine HCl, and yohimbine HCl (Sigma); phentolamine mesylate and naphazoline HCl (Ciba-Geigy); prazosin HCl (Pfizer); indoramin (Wyeth) (±)-α-methylnorepinephrine (Sterling-Winthrop); piperoxan HCl (May and Baker); BE 2254 (2-[β-(4-hydroxyphenyl)-ethylaminomethyl]-tetralone) (Beiersdorf AG); ARC 239 (2-[2,4-(*O*-methoxyphenyl)-piperazin-1-yl] ethyl-4,4 dimethyl-1,3-(2*H*,4*H*)-isoquinolindione dihydrochloride) and tramazoline HCl (Dr. Karl Thomae GMBH); nylidrin HCl and desipramine HCl (USV Pharmaceutical Corporation); clonidine HCl and *p*-aminoclonidine (Boehringer Ingelheim); isoxsuprine HCl (Mead Johnson); oxymetazoline HCl (Schering Corporation); azapetine phosphate (Hoffmann-La Roche), ephedrine sulfate, tolazoline HCl (courtesy Dr. N. C. Moran, Department of Pharmacology, Emory University Medical School); methoxamine (Burroughs Wellcome); and (±)-propranolol (Ayerst Laboratories).

Preparation of rat vas deferens for binding assays. Male Sprague-Dawley rats (175–300 g) were killed by cervical dislocation and the vasa deferentia were removed and placed on ice without removing the connective tissue sheath. A pair of vasa deferentia from a single animal (125–175 mg wet weight) was homogenized in 20 ml 20 mM $NaPO_4$ buffer (pH 7.6) containing 154 mM NaCl (PO_4-salt buffer) with a Polytron at speed 7 for 15 sec. The homogenate was centrifuged at 20,000 × *g* for 10 min, the supernatant was discarded, and the pellet was resuspended in PO_4-salt buffer to a final tissue concentration of 20 mg (wet weight) per milliliter. The homogenate was then filtered through a double layer of surgical gauze to remove connective tissue fragments, and used for the binding assays without further preparation.

Measurement of ^{125}IBE binding. BE 2254 was radioiodinated to theoretical specific activity as described by Engel and Hoyer (13) and stored for up to 1 month at −20° in methanol. Measurement of specific ^{125}IBE binding was usually performed by incubating 100 μl of tissue homogenate (20–40 μg of protein) with ^{125}IBE (usually 30,000 cpm; 50 pM), 20 mM $NaPO_4$ (pH 7.6), 154 mM NaCl, and competing drugs in a final volume of 250 μl for 20 min at 37°. At the end of the incubation period, each tube was diluted with 10 ml of 10 mM Tris-HCl (pH 7.4) at 20° and immediately poured over a glass-fiber filter (Schleicher and Schuell No. 30) under reduced pressure. Each filter was washed with 10 ml of 10 mM Tris-HCl (pH 7.4), dried, and counted in minivials in a scintillation counter at 50% efficiency. Receptor binding was determined by the difference between binding in the absence of competing drugs and binding in the presence of 10^{-5} M phentolamine, and was usually about 70% of total binding.

The potency of various drugs in competing for the specific ^{125}IBE binding sites was determined by incubating ^{125}IBE (around 50 pM) in the presence or absence of 16 concentrations of competing drug. EC_{50} values for competing drugs were determined as the *x*-intercept on a Hill plot, and K_D values were calculated from EC_{50} values by the method of Cheng and Prusoff (19). Saturation curves were determined by incubating tissue with increasing concentrations of ^{125}IBE (20–400 pM) in the presence or absence of 10^{-5} M phentolamine and analyzing the data by the method of Scatchard (20).

Measurement of contractile responses. Male Sprague-Dawley rats (175–300 g) were pretreated with reserpine (3 mg/kg i.p.). Animals exhibiting ptosis 16–20 hr later were killed, and the vasa deferentia were removed and placed in KRB buffer containing 120 mM NaCl, 5.5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 20 mM $NaHCO_3$, 11 mM glucose, and 0.029 mM $CaNa_2$ EDTA and gassed with 95% O_2:5% CO_2. The KRB buffer also contained 0.1 μM desmethylimipramine to block neuronal uptake of norepinephrine, 1.0 μM (±)-propranolol to block beta-adrenergic receptors, and 1.0 μM normetanephrine to block extraneuronal uptake. Each vas deferens was carefully dissected free of its adherent sheath of connective tissue and suspended in an organ bath of either 5 ml or 10 ml volume, maintained at 37°, and attached to a Grass FT.03 force displacement transducer. The preparations were stretched to a resting tension of 400 mg and allowed to equilibrate for 1 hr in KRB buffer, with continuous readjustment of resting tension to 400 mg. At the end of the equilibration period the tissues were exposed twice to a maximal concentration of norepinephrine and thoroughly washed.

Dose-response curves for increases in isometric tension were determined according to a noncumulative dosage schedule where each dose was washed out before the

next dose was added, using 6-min intervals between doses. pA_2 values for antagonists (21) were calculated by first performing dose-response curves for an agonist in the absence of antagonist, adding antagonist to the bathing solution in three or four increasing concentrations, and repeating the dose-response curve to the agonist. Each new dose of antagonist was allowed to equilibrate for at least 45 min before performing the agonist dose-response curve. Responses were measured as the peak tension developed while the preparation was exposed to the agonist, and are expressed as a percentage of the maximal contraction induced by norepinephrine or phenylephrine. EC_{50} values for each log dose-response curve were calculated by linear regression of all points between 20% and 80% of the maximal response to that agonist. Previous data[3] demonstrated that the reserpine pretreatment schedule used here has no effect on the affinity or efficacy of norepinephrine in producing contractions of rat vas deferens.

RESULTS

Binding of ^{125}IBE to alpha$_1$-adrenergic receptors in rat vas deferens. The characteristics of ^{125}IBE binding to homogenates of rat vas deferens are shown in Fig. 1. Specific receptor binding of ^{125}IBE was determined to be binding-displaceable by 10 μM phentolamine. Specific binding was saturable and of high affinity, and Scatchard analysis of saturation isotherms resulted in a K_D of 105 ± 15 pM and a B_{max} of 215 ± 30 fmoles/mg of protein (n = 5). Nonreceptor binding was usually around 30% of total binding at the lowest concentrations of ^{125}IBE (50 pM or less) and was linear with increasing radioligand concentration. The kinetic mechanisms of association and dissociation of specific ^{125}IBE binding in homogenates of rat vas deferens are shown in Fig. 2. Association was very rapid at 37°, and equilibrium conditions were established within 15 min. The pseudo-first order plot for association gave a k_1 of 3.8×10^7 1/mole-sec. The binding of ^{125}IBE was reversed by addition of a large excess of phentolamine (40 μM), and the first-order plot of dissociation gave a k_{-1} of 2.1×10^{-3} sec^{-1} at 37°. Thus, the kinetically determined K_D (60 pM) is in reasonable agreement with the K_D determined by equilibrium analysis (105 pM). These constants also agree fairly well with values published previously for ^{125}IBE binding to rat brain membranes (13, 14).

Inhibition of ^{125}IBE binding by alpha-adrenergic antagonists. Specific binding of ^{125}IBE to membranes of rat vas deferens was inhibited by a variety of alpha-adrenergic receptor antagonists (Table 1). The order of potency of these compounds is similar to that reported previously in rat brain membranes (13, 14) and is similar to that expected of an *alpha*$_1$-adrenergic receptor. All drugs were able to inhibit specific ^{125}IBE binding completely in membranes from rat vas deferens, and the calculated Hill coefficients suggested that all of these drugs, except azapetine, inhibited ^{125}IBE binding in a noncooperative

[3] K. P. Minneman, A. W. Fox, and P. W. Abel, unpublished observations.

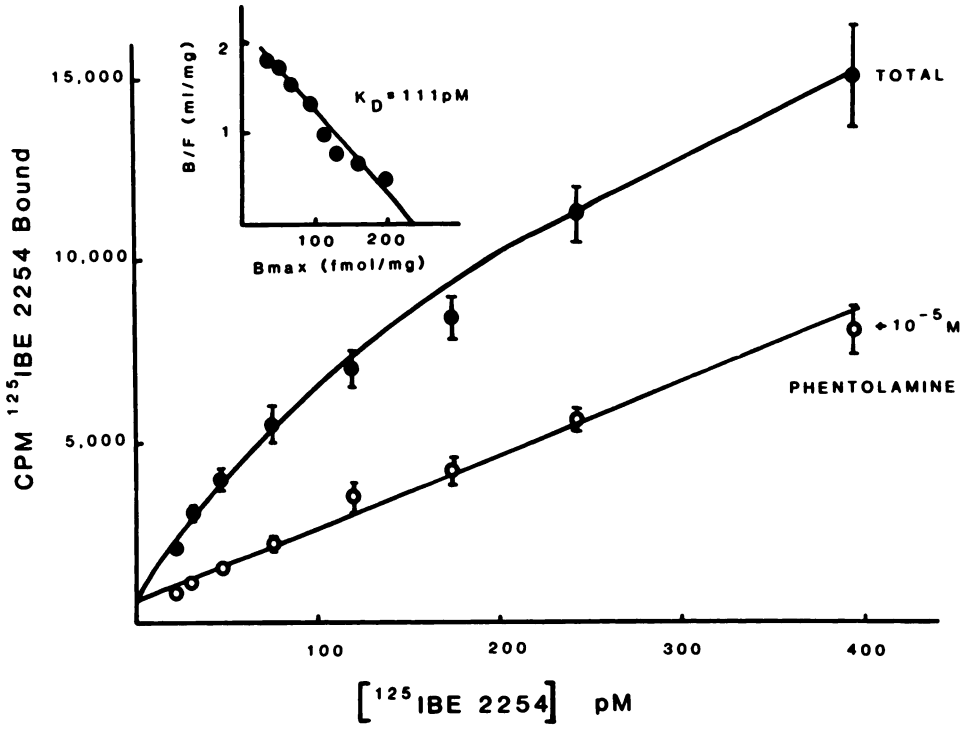

FIG. 1. *Binding of ^{125}IBE to membrane fragments from rat vas deferens*

Membranes were prepared from rat vas deferens as described in the text, and an aliquot was incubated with varying concentrations of ^{125}IBE in the presence of 20 mM $NaPO_4$ (pH 7.6) and 154 mM NaCl for 20 min at 37° in the absence (●) or presence (○) of 10 μM phentolamine. *Inset*, Specific ^{125}IBE binding (defined as the difference between binding in the absence and presence of phentolamine) plotted by the method of Scatchard (20). The values on the *y*-axis (*B/F*, bound/free) were determined by dividing the amount bound (femtomoles per milligram of protein) by the concentration of free radioligand (femtomoles per milliliter). At the lowest concentration of ^{125}IBE, 5.7% of the radioligand was bound in this experiment. Each point represents the mean ± standard error of the mean of three replicates from a single experiment.

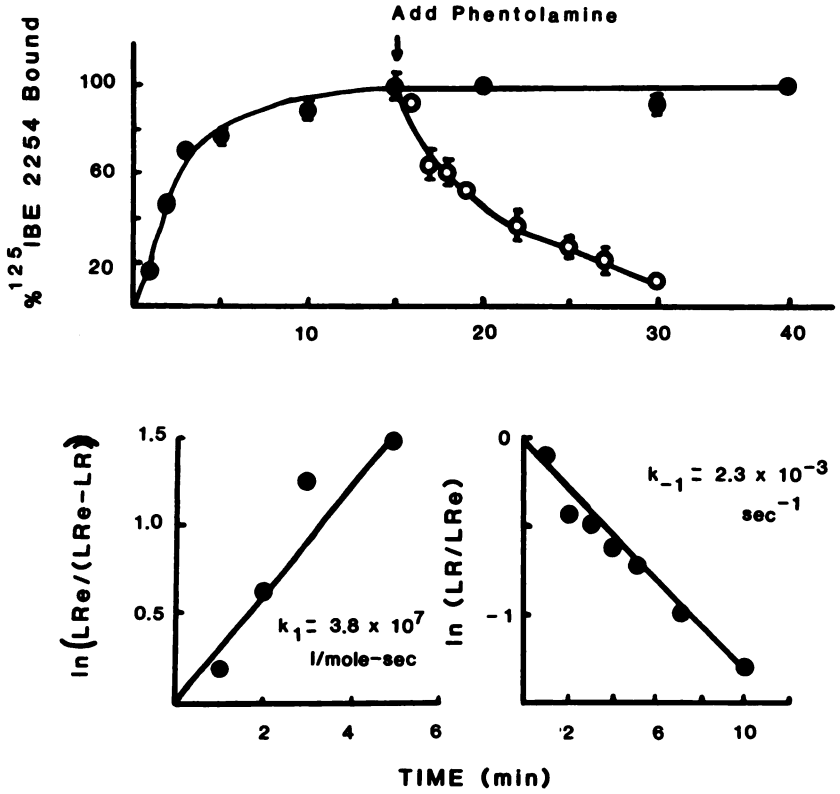

FIG. 2. *Time dependence for association and dissociation of ^{125}IBE with membranes from rat vas deferens*

Top, Specific ^{125}IBE binding (94 pM) plotted as a function of time of incubation at 37°. ●, Total specific binding; ○, specific binding remaining after the addition of 40 μM phentolamine. Scatchard analysis of equilibrium binding data obtained with the homogenate used in this experiment gave a total receptor density (B_{max}) of 1.71 fmoles/250 μl (6.84 pM) and a K_D of 96 pM for ^{125}IBE.

Bottom left, Pseudo-first order plot of association. The k_1 was obtained by using the equation $k_1 = k_{obs} - RLe/L_T \cdot R_T$, where k_{obs} is the slope of the pseudo-first order plot. Bottom right, First-order plot of dissociation. Each point represents the mean ± standard error of the mean of three replicates from a single experiment.

manner, with no evidence for binding heterogeneity (Table 1). The Hill coefficient for inhibition of ^{125}IBE binding by azapetine was significantly less than 1.0; the reason for this is unknown (see Discussion).

pA_2 Values for antagonists in inhibiting contractile response of rat vas deferens. The effects of alpha-adrenergic agonists in causing contraction of rat vas deferens might be mediated through either *alpha*$_1$- or *alpha*$_2$-adrenergic receptors, or through both subtypes. Therefore, pA_2 values for *alpha*$_1$- and *alpha*$_2$-selective antagonists in competitively inhibiting the response to phenylephrine (which stimulates only *alpha*$_1$-receptors) were compared with the pA_2 values against norepinephrine and epinephrine (which stimulate both *alpha*$_1$- and *alpha*$_2$-receptors). All drugs tested, including both *alpha*$_1$-selective antagonists (prazosin, BE 2254, indoramin, and ARC 239) and *alpha*$_2$-selective antagonists (piperoxan and yohimbine) competitively inhibited the contractile response to norepinephrine, epinephrine, and phenylephrine (Fig. 3). Comparison of the pA_2 values for each selective antagonist against each of the three agonists (Table 2) suggested that phenylephrine, epinephrine, and norepinephrine were causing their contractile effects by interacting with the same homogeneous population of *alpha*-adrenergic receptors in rat vas deferens, and that these receptors had the pharmacological characteristics of *alpha*$_1$-adrenergic receptors. We obtained no evidence for an *alpha*$_2$-adrenergic receptor-mediated contraction in this tissue.

Comparison of antagonist pA_2 values with K_D values. In order to determine whether ^{125}IBE was labeling the *alpha*$_1$-adrenergic receptors mediating the contractile response in rat vas deferens, the pA_2 value of antagonists in competitively inhibiting the contractile response to

TABLE 1

Equilibrium dissociation constants (K_D values) for inhibition of ^{125}IBE binding in rat vas deferens by alpha-adrenergic receptor antagonists

^{125}IBE (45–55 pM) was incubated with 16 concentrations of each drug. Nonspecific binding was determined as ^{125}IBE binding remaining in the presence of 10 μM phentolamine. Hill plots were constructed for the inhibition of ^{125}IBE binding by each drug, and IC_{50} values and Hill coefficients (n_H) were determined by linear regression. K_D values were calculated from IC_{50} values by the method of Cheng and Prusoff (19). Each value represents the mean ± standard error of the mean of three to five determinations.

Drug	K_D	n_H
	nM	
Prazosin	0.6 ± 0.1	1.16 ± 0.097
BE 2254	1.2 ± 0.4	1.14 ± 0.059
Indoramin	14.0 ± 2.7	1.09 ± 0.089
ARC 239	14.0 ± 8.0	0.99 ± 0.062
Phentolamine	26.0 ± 6.0	1.11 ± 0.076
Azapetine	57.0 ± 14.0	0.78 ± 0.080
Piperoxan	900.0 ± 121	1.05 ± 0.051
Yohimbine	1070.0 ± 157	1.10 ± 0.117
Tolazoline	1900.0 ± 320	0.92 ± 0.026

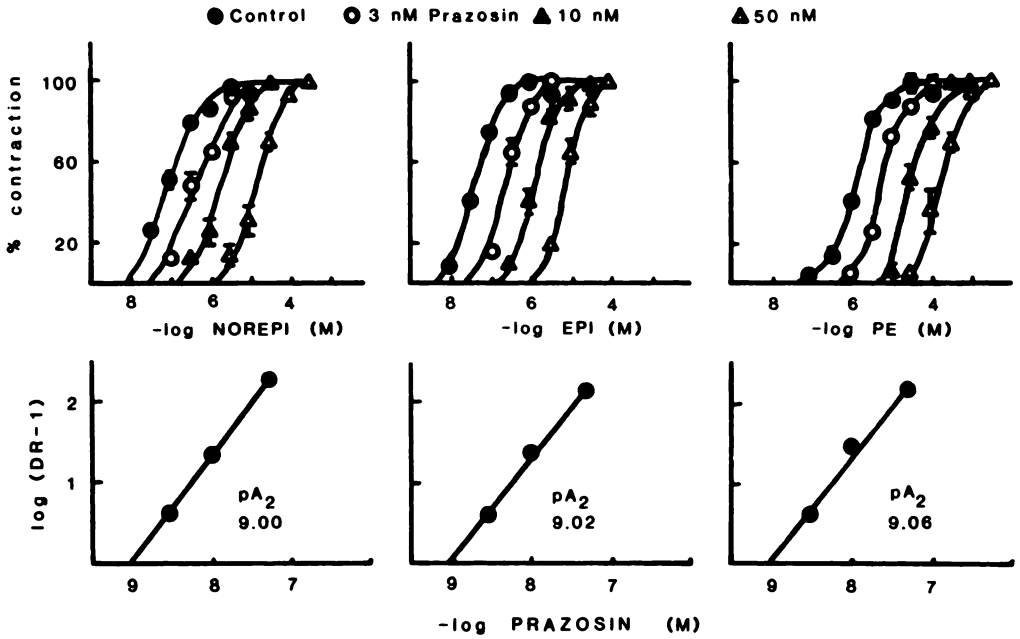

FIG. 3. *Effect of prazosin on contractions of rat vas deferens induced by norepinephrine (NOREPI), epinephrine (EPI), and phenylephrine (PE)*

Top, Noncumulative dose-response curves to each agonist were determined in the absence of prazosin (●) or after 45 min of equilibration with 3 nM (○), 10 nM (▲), or 50 nM (△) prazosin.

Bottom, Schild plots (21) of the above data. Plotted on the *y*-axis is the log of the dose ratio (*DR*) calculated as the EC_{50} for the agonist in the presence of competing drug divided by the EC_{50} for the agonist in the absence of competing drug, minus 1. pA_2 values were determined by linear regression and did not differ significantly between the three agonists. Each point represents the mean ± standard error of the mean of four determinations on separate preparations.

norepinephrine was compared with the K_D value for the same drugs in inhibiting ^{125}IBE binding (Fig. 4). An excellent correlation (r = 0.995) was observed between the two parameters.

Comparison of the effects of full agonists on contractile responses and ^{125}IBE binding in rat vas deferens. Dose-response curves for the effects of epinephrine, norepinephrine, phenylephrine, and methoxamine in increasing contraction and inhibiting ^{125}IBE binding in rat vas deferens are shown in Fig. 5. All of these full agonists (adrenergic agonists giving a maximal contraction equal to norepinephrine on the vas deferens) were 10- to 100-fold more potent in causing the functional response than they were in inhibiting ^{125}IBE binding (Table 3), possibly due to a population of "spare" *alpha*-adrenergic receptors in this tissue (22).

Comparison of the effect of partial agonists on contractile responses and ^{125}IBE binding in rat vas deferens. Dose-response curves for the effects of partial agonists (those agonists which caused a smaller maximal contraction than did norepinephrine) in increasing con-

TABLE 2

Comparison of pA_2 values for selective alpha-adrenergic blocking agents against phenylephrine, norepinephrine and epinephrine-induced contractions in rat vas deferens

Dose-response curves for generation of isometric tension by each agonist were determined in the absence or presence of three or four concentrations of each antagonist. EC_{50} values were determined by linear regression between 20% and 80% of maximal response and pA_2 values calculated (21).

	pA_2 Value against		
	Phenylephrine	Norepinephrine	Epinephrine
Prazosin	8.95 ± 0.07	8.97 ± 0.02	9.03 ± 0.09
BE 2254	8.97 ± 0.07	8.81 ± 0.24	9.15 ± 0.13
Indoramin	7.56 ± 0.09	7.79 ± 0.09	7.49 ± 0.02
ARC 239	7.68 ± 0.04	7.88 ± 0.15	8.14 ± 0.13
Piperoxan	5.90 ± 0.12	5.98 ± 0.03	6.31 ± 0.33
Yohimbine	6.22 ± 0.12	6.13 ± 0.30	5.40 ± 0.11

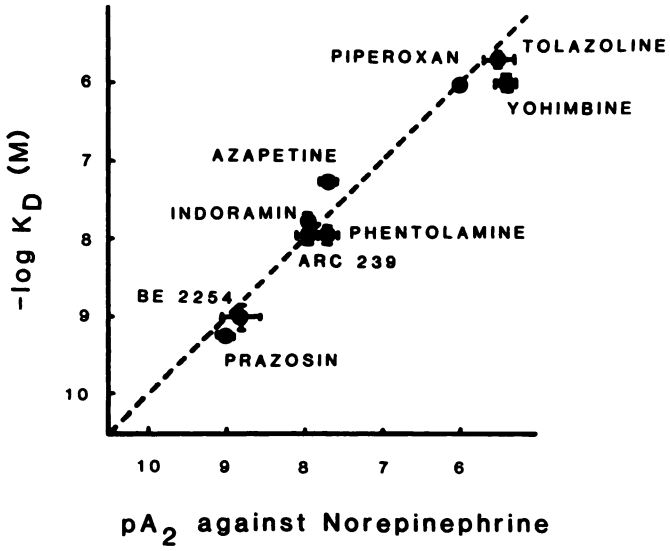

FIG. 4. *Comparison of the pA_2 values of antagonists in competitively inhibiting norepinephrine induced contractions and the K_D values for inhibiting ^{125}IBE binding*

Each point represents the mean ± standard error of the mean for three to eight determinations on different tissues. The *dashed line* represents theoretical agreement.

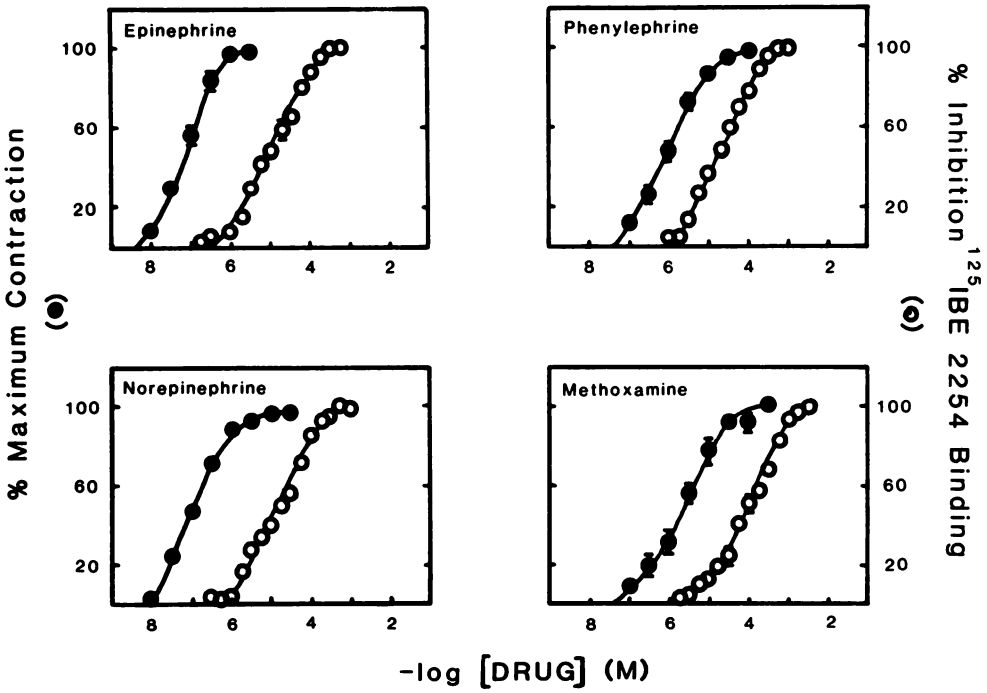

FIG. 5. *Comparison of the dose-response curves for full agonists in increasing contraction and inhibiting ^{125}IBE binding in rat vas deferens*
Dose-response curves for each agonist in increasing contraction (●) and inhibiting ^{125}IBE binding (○) were determined as described in the text. Each point represents the mean ± standard error of the mean of three to eight determinations on separate preparations.

traction and inhibiting ^{125}IBE binding in rat vas deferens are shown in Fig. 6. In general, there was a good correlation between the EC_{50} values for increasing contraction and the K_D values for inhibiting ^{125}IBE binding in this tissue (Table 4). The effects of all partial agonists in causing contraction were also tested before and after equilibration of the tissue with 20 nM prazosin. In each case this concentration of prazosin caused a 10- to 20-fold shift to the right in the dose-response curves for the partial agonists (data not shown), indicating that these drugs are causing their contractile effects through *alpha*$_1$-receptors.

Effect of incubation conditions on characteristics of ^{125}IBE binding sites. The apparent binding characteristics of many agonists to their receptors in radioligand binding assays are markedly dependent on the conditions of the incubation, such as the presence or absence of cations or guanine nucleotides (see ref. 10). For this reason, we determined whether the apparent K_D values for agonists in inhibiting ^{125}IBE binding were affected by NaCl, $MgCl_2$, or GTP. When binding assays were performed in 14 mM KPO_4 buffer (pH 7.6), there was no significant effect of adding 154 mM NaCl, 10 mM $MgCl_2$, or 100 μM GTP on the density of ^{125}IBE binding sites, the K_D value for ^{125}IBE (Table 5), or the K_D value for norepinephrine in displacing ^{125}IBE binding in this tissue. The K_D values for norepinephrine in displacing ^{125}IBE binding were 10.3 ± 5.5 μM in the absence of NaCl and $MgCl_2$ and 7.2 ± 2.4 μM in the presence of 154 mM NaCl and 10 mM $MgCl_2$ (mean ± standard error of the mean of three determinations). In order to control for possible degradation of catecholamines during the binding assays, dose-response curves to norepinephrine and epinephrine were also performed in the presence or absence of 40 μM pargyline (to inhibit monoamine oxidase), 40 μM pyrogallol (to inhibit catechol-*O*-methyltransferase), 100 nM desmethylimipramine (to inhibit neuronal uptake), 1 mM ascorbic acid, or all of these drugs. There was no effect of any single drug or a combination of these drugs on the apparent K_D values for norepinephrine or epinephrine in inhibiting ^{125}IBE binding (data not shown).

TABLE 3

Comparison of K_D values for inhibition of ^{125}IBE binding and EC_{50} values for contractile responses for full agonists in rat vas deferens

K_D values for inhibition of ^{125}IBE binding and EC_{50} values for increases in isometric tension were determined as described in the text.

	EC_{50} for contraction	K_D for binding	Ratio (K_D/EC_{50})
	μM	μM	
Norepinephrine	0.12 ± 0.013	11.2 ± 0.76	93.3
Epinephrine	0.09 ± 0.014	7.4 ± 0.51	82.2
Methoxamine	2.9 ± 0.92	72.9 ± 6.7	25.0
Phenylephrine	1.4 ± 0.28	13.1 ± 1.1	9.2
(±)-α-methylnorepinephrine	3.2 ± 0.82	44.5 ± 17.1	13.9

Competitive interaction of agonists with ^{125}IBE binding sites. To show that epinephrine was competitively inhibiting ^{125}IBE binding, Scatchard analysis of specific ^{125}IBE binding was measured in the presence of increasing concentrations of epinephrine (Fig. 7). Epinephrine caused a dose-dependent increase in the apparent K_D for ^{125}IBE with no change in the B_{max}, indicating a true competitive interaction. Hill coefficients for the inhibition of ^{125}IBE binding by all agonists and partial agonists were close to 1.0, indicating a simple mass-action competition, with the exception of oxymetazoline (0.60 ± 0.085; $n = 3$) and tramazoline (0.69 ± 0.049; $n = 3$) which had low Hill coefficients. Other imidazolines, such as clonidine and naphazoline, had Hill coefficients close to

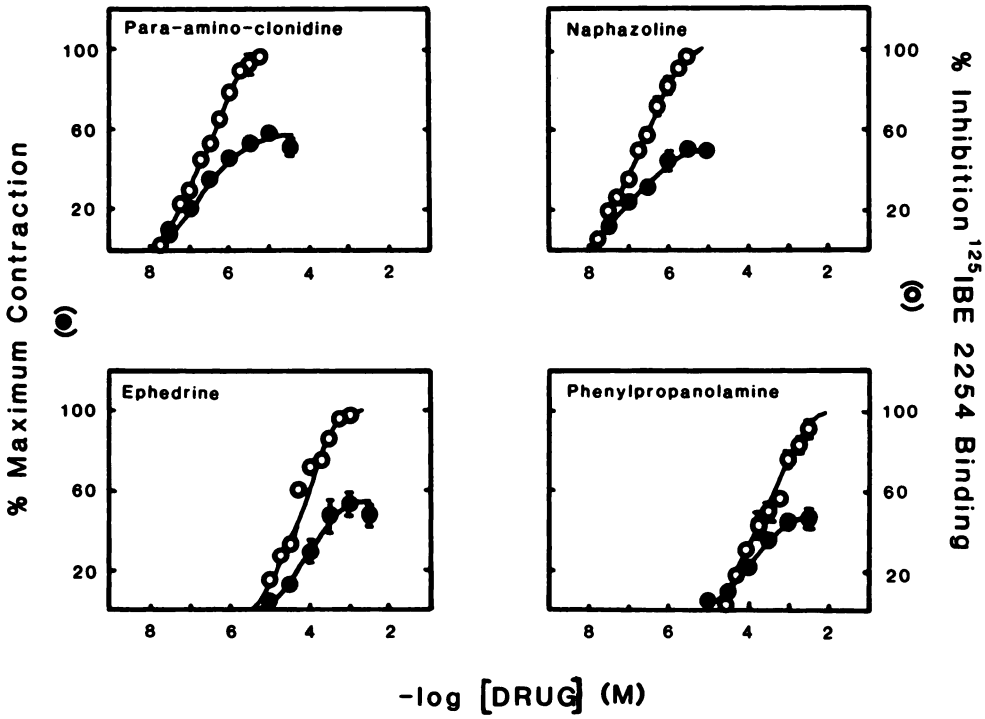

FIG. 6. *Comparison of the dose-response curves for partial agonists in increasing contraction and inhibiting ^{125}IBE binding in rat vas deferens*

Dose-response curves for each partial agonist in increasing contraction (●) and inhibiting ^{125}IBE binding (○) were determined as described in the text. Each point represents the mean ± standard error of the mean of three or four determinations on separate preparations.

1.0 (data not shown). The reason for these differences is presently unknown (see Discussion).

DISCUSSION

Several radiolabeled compounds are now in use for the direct labelling and identification of *alpha*-adrenergic receptor subtypes (8). Many of these compounds have a high affinity and specificity for binding sites in membranes from rat brain and other tissues which have pharmacological properties similar to those of *alpha*-adrenergic receptors. As such, these compounds have been used to examine the density and properties of putative *alpha*-adrenergic receptor subtypes in various tissues under a variety of conditions. However, little information is yet available concerning the relationship between receptor occupancy and tissue response, which will only come from a direct comparison, in the same tissue, of the affinity of drugs for *alpha*-adrenergic receptor binding sites and their potency in activating or inhibiting the functional response of that tissue.

In this study we have attempted to correlate the occupancy of *$alpha_1$*-adrenergic receptors in rat vas deferens with the receptor-mediated contractile response. ^{125}IBE has previously been shown to label binding sites with properties similar to those of *$alpha_1$*-adrenergic receptors in membranes from rat brain (13, 14). We show

TABLE 4

Comparison of K_D values for inhibition of ^{125}IBE binding and EC_{50} values for contractile responses for partial agonists in rat vas deferens

K_D values for inhibition of ^{125}IBE binding and EC_{50} values for increases in isometric tension were determined as described in the text.

	EC_{50} for contraction	K_D for binding	Intrinsic activity
	μM	μM	% of norepinephrine
Oxymetazoline	0.14 ± 0.018	0.19 ± 0.020	72 ± 1.2
Naphazoline	0.14 ± 0.105	0.14 ± 0.003	50 ± 2.9
p-Aminoclonidine	0.21 ± 0.114	0.28 ± 0.095	58 ± 3.1
Tramazoline	0.75 ± 0.053	0.14 ± 0.020	68 ± 3.3
Clonidine	0.75 ± 0.220	0.40 ± 0.070	62 ± 2.2
Ephedrine	78.0 ± 7.0	36.0 ± 1.0	52 ± 6.3
Phenylpropanolamine	181.0 ± 45.0	220.0 ± 41.0	47 ± 3.3

TABLE 5

Effect of incubation conditions on the binding of ^{125}IBE to membranes from rat vas deferens

Scatchard analysis of specific ^{125}IBE binding was determined, as described in the text, in 14 mM KPO_4 buffer (pH 7.6) containing the various substances indicated. Each point represents the mean ± standard error of the mean of determinations from three different animals.

Additional factors added to incubation	K_D	B_{max}
	pM	fmoles/mg protein
None	89 ± 13	190 ± 10
154 mM NaCl	96 ± 22	215 ± 18
154 mM NaCl	91 ± 25	209 ± 17
154 mM NaCl + 10 mM $MgCl_2$	79 ± 6	200 ± 48
154 mM NaCl	88 ± 5	176 ± 32
154 mM NaCl + 100 μM GTP	96 ± 14	188 ± 20

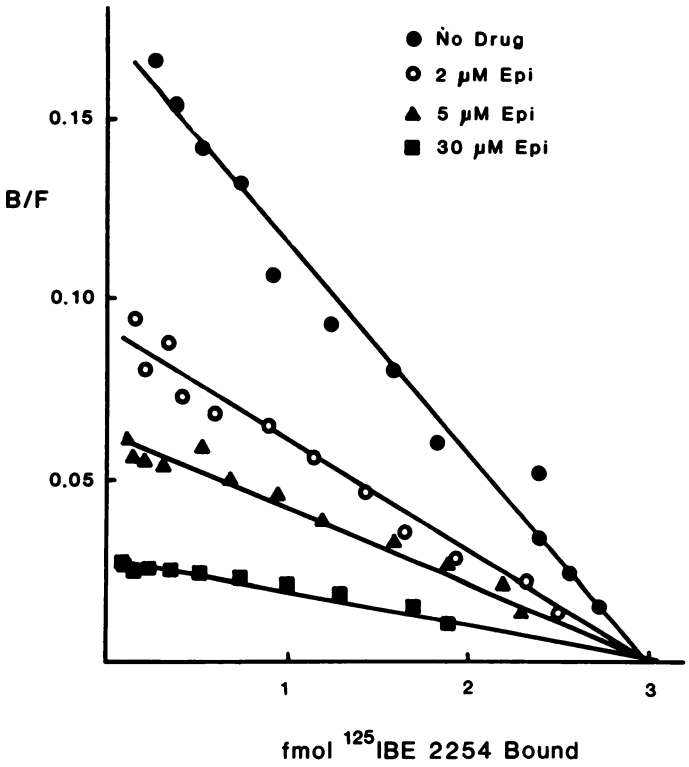

FIG. 7. *Scatchard analysis of* ^{125}*IBE binding to rat vas deferens in the presence of* 0 (●), 2 μM (○), 5 μM (▲), *or* 30 μM (■) *epinephrine* (*Epi*)

Experimental details as described in the text. Each point represents the mean of duplicate determinations.

here that ^{125}IBE labels binding sites in rat vas deferens with properties similar to those of *alpha*$_1$-adrenergic receptors, and that the kinetic and equilibrium properties, as well as the pharmacological specificity, of these binding sites are similar to those observed previously in rat brain membranes (13, 14). However, if less than 10% of the receptors labeled by ^{125}IBE in this tissue were *alpha*$_2$-receptors, then we would be unable to distinguish this population with our binding experiments.

Substantial evidence now indicates that *alpha*$_1$- and *alpha*$_2$-adrenergic receptors can coexist in some smooth muscles and that both receptor subtypes mediate contraction (3, 4). For this reason, it was important to determine whether the norepinephrine-induced contraction of rat vas deferens was mediated by either *alpha*$_1$- or *alpha*$_2$-receptors or by both receptor subtypes. Comparison of the pA_2 values for *alpha*$_1$- and *alpha*$_2$-selective antagonists in competitively inhibiting the contractile responses to norepinephrine and epinephrine (which act on both *alpha*$_1$- and *alpha*$_2$-receptors) and to phenylephrine (which acts only on *alpha*$_1$- receptors) showed that all three of these drugs were acting through a similar and apparently homogeneous receptor population in this tissue. The order of potency of antagonists in inhibiting this response suggested that these receptors should be classified as *alpha*$_1$-adrenergic receptors. We obtained no evidence for the existence of postsynaptic *alpha*$_2$-adrenergic receptors mediating contraction in rat vas deferens, although previous evidence suggests that presynaptic *alpha*$_2$-receptors do exist on sympathetic nerve terminals and alter transmitter release in this tissue (18).

Measuring both *alpha*$_1$-adrenergic receptor occupancy by inhibition of specific ^{125}IBE binding and norepinephrine-induced contractile responses in the same tissue allows a direct correlation between receptor occupancy and tissue response. An excellent correlation was observed between the pA_2 values for selective antagonists in competitively inhibiting the norepinephrine-induced contractions and the K_D values of the same drugs in inhibiting specific ^{125}IBE binding. This provides good evidence that the binding sites labeled by ^{125}IBE are identical with the functional *alpha*-adrenergic receptor in this tissue. Schmitz *et al.* (23) have compared the affinity of agonists and antagonists to inhibit [^{3}H]prazosin binding to *alpha*$_1$-adrenergic receptors in rat renal membranes and their ability to stimulate or inhibit vasoconstriction in the isolated perfused rat kidney. A good correlation between the affinity of antagonists to inhibit [^{3}H]prazosin binding and their potency in the isolated perfused kidney was obtained; however, agonists were generally more potent in activating contraction than in competing for the [^{3}H]prazosin binding site. These results are similar to those reported here in the rat vas deferens. In addition, it has been reported previously that the binding sites in rat brain selectively labeled by [^{3}H]prazosin and [^{3}H]WB 4101 have the same antagonist selectivity as *alpha*-adrenergic receptor-mediated functional responses in various peripheral vascular beds (15, 16), although the binding and functional data were not obtained from the same tissues. In contrast, Holck *et al.* (24) compared the binding properties of *alpha*-adrenergic drugs by their ability to displace the putative *alpha*-receptor ligand [^{3}H]dihydroergocryptine from membranes of guinea pig vas deferens with the effects of these drugs in activating or inhibiting contraction in this tissue; however, no good correlation was observed. This may be due to the relatively nonselective nature of [^{3}H]dihydroergocryptine, which binds to both *alpha*$_1$- and *alpha*$_2$-receptors as well as dopaminergic and serotonergic receptors (25), to heterogeneity in the receptor population of guinea pig vas deferens, or to other factors.

The affinities of full agonists in inhibiting ^{125}IBE binding in rat vas deferens did not correlate well with their potency in inducing a contractile response in this tissue. All full agonists tested were 10- to 100-fold more potent in activating contraction than in inhibiting ^{125}IBE binding. This difference was not unexpected, since experimental evidence based on the use of irreversible receptor-alkylating agents has suggested that there may be a large pool of "spare" *alpha*-adrenergic receptors in some smooth muscles (22, 26–28). According to this hypothesis, there are many more receptors in some tissues than are necessary to provoke a maximal functional response, and only a small proportion of receptors need be occupied by agonist to obtain a maximal response. These "spare" receptors would increase the apparent potency of the agonist in causing a response, since by the law of mass action lower concentrations of agonist would be necessary to occupy a smaller proportion of receptors to obtain a maximal response. Thus, this theory predicts a nonlinear relationship between receptor occupancy and tissue response for full agonists as observed in the present study. In addition, this hypothesis also predicts a more nearly linear relationship between receptor occupancy

and the functional response to partial agonists. The good agreement between receptor occupancy and tissue response for partial agonists in the present study is consistent with the "spare" receptor theory.

It is important to point out, however, that the affinity of agonists for various receptors when measured with radioligand binding assays are often markedly affected by the conditions of the binding assay. The presence or absence of guanine nucleotides or cations can alter the binding properties of various agonists, particularly for receptors which are linked to activation or inhibition of adenylate cyclase (see ref 10). It is therefore important to determine that the apparent affinity of agonists in inhibiting ^{125}IBE binding reflects the true occupancy of *alpha*$_1$-adrenergic receptors. Although one report (29) has shown large effects of divalent cations on ^{125}IBE binding to brain membranes, in our experiments we could find no significant effect of Na^+, Mg^{2+}, or GTP on either ^{125}IBE binding or the potency of norepinephrine in inhibiting ^{125}IBE binding. This was not surprising, since *alpha*$_1$-adrenergic receptors are not thought to be directly linked to adenylate cyclase (30) and most other studies have also found no effect of GTP, Mg^{2+}, or Na^+ on the affinity of agonists for these receptors (11, 31). In addition, there was no effect of desmethylimipramine, pargyline, pyrogallol, or ascorbic acid on the potency of norepinephrine in inhibiting ^{125}IBE binding, suggesting that uptake or degradation of catecholamines in the binding assays is not causing a decrease in apparent potency. We have also shown that the inhibition of ^{125}IBE binding by epinephrine, norepinephrine, and other agonists is purely competitive when determined by Scatchard analysis. Thus, in contrast to the complexity of agonist binding to many other receptors, at this time it appears that agonist binding to *alpha*-adrenergic receptors is relatively simple, and is not consistently affected by any experimental conditions yet examined.

It is impossible to state with certainty, however, that the dose-response relationship of agonists in inhibiting ^{125}IBE binding in membrane fragments of rat vas deferens is identical with the dose-response relationship for occupation of *alpha*-adrenergic receptors in the intact vas deferens. To obtain experimental proof of this relationship would require performing binding assays on intact vasa deferentia under identical conditions for contraction, a procedure which is currently technically impossible. Alternatively, if spare *alpha*$_1$-receptors do exist in rat vas deferens, one could directly examine the spare receptor pool using irreversible alkylating agents such as phenoxybenzamine, and correlate direct measurement of receptor density with alterations in the dose-response curve for agonist-induced contractions. In this manner, true K_A values for agonists in causing contraction could be determined by the method of Furchgott (26) and directly compared with the K_D values for binding. These experiments are currently in progress. However, previously reported K_A values for norepinephrine interaction with *alpha*-adrenergic receptors in rabbit aorta and spleen (approximately 0.3 μM) (27, 28) are similar to the EC_{50} values reported here in the vas deferens and are substantially lower than the K_D against ^{125}IBE binding. Although it is not clear what types of *alpha*-adrenergic receptors were present in the aorta and spleen in the previous studies, these data might indicate a discrepancy between true K_A values for contraction and K_D values determined in binding assays. Ariens *et al.* (22) have presented evidence that rat vas deferens contains few, if any, spare receptors, suggesting that one might expect good agreement between K_D values for binding and EC_{50} values for contraction in this tissue. If there are few spare receptors in rat vas deferens, then a discrepancy between K_A and K_D might be due to a rapid desensitization process, differences between the binding properties in whole cells and broken cells, lack of essential cofactors in the binding assays, or other unknown parameters. On the other hand, since the EC_{50} values of partial agonists in contracting rat vas deferens correlate well with the K_D values for inhibition of ^{125}IBE binding, the discrepancies observed for full agonists may represent spare receptor pools. These questions are currently under investigation.

There is some controversy concerning whether the *alpha*$_1$-adrenergic receptors mediating the contractile response to norepinephrine in rat vas deferens comprise a homogeneous population. Ruffolo *et al.* (32) reported a lack of cross-desensitization between imidazolines and phenylethanolamines in rat vas deferens, and McGrath (33) reported an anatomical separation in the excitatory effects of oxymetazoline in the two ends of the rat vas deferens. Although our results indicate a homogeneous population of *alpha*$_1$-adrenergic receptors in the rat vas deferens, we cannot completely rule out a possible heterogeneity. Indeed, the agonists oxymetazoline and tramazoline, and the antagonist azapetine all had low Hill coefficients for inhibiting ^{125}IBE binding, suggesting some complexity in binding or heterogeneity in receptor population. However, the dose ratios for oxymetazoline or tramazoline for increasing contraction in the absence or presence of 20 nM prazosin did not significantly differ from the dose ratios for other agonists under these conditions (data not shown), suggesting that all of these drugs are acting on prazosin-sensitive *alpha*$_1$-receptors. Further experiments are necessary to clarify this issue.

In summary, we have presented evidence that rat vas deferens contains an apparently homogeneous population of *alpha*$_1$-adrenergic receptors mediating the contractile response to norepinephrine, that these receptors can be labeled with ^{125}IBE, and that there is an apparently nonlinear relationship between the occupancy of these receptors and the functional response of the tissue.

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