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# Initial Detection of [$^3$H]Prazosin-Labeled *Alpha*$_1$-Receptors in the Porcine Pituitary Neurointermediate Lobe

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SUMMARY

[$^3$H]Prazosin binding to *alpha*$_1$-adrenergic receptors was investigated in homogenates of tissue from the porcine pituitary neurointermediate lobe. Potent *alpha*$_1$-adrenergic antagonists such as prazosin, WB-4101, and phentolamine displayed high potency in competing for [$^3$H]prazosin specific binding in pituitary tissue. Epinephrine and norepinephrine were the only neurotransmitters which displayed high potency in competing for [$^3$H]prazosin binding sites. There was a strong correlation in the pharmacological properties of the *alpha*$_1$ sites in pituitary tissue and the *alpha*$_1$ sites detected by [$^3$H]prazosin in rat brain tissue. *Alpha*-adrenergic agonists demonstrated significantly higher potency for the *alpha*$_1$-receptor sites in pituitary tissue than in brain tissue preparations. The antagonist ketanserin demonstrated significantly higher potency at *alpha*$_1$ sites in pituitary tissue than in brain tissue. This latter observation has important implications for investigators attempting to use [$^3$H]ketanserin as a selective serotonin receptor ligand in pituitary tissue. The results of this study represent the first demonstration of *alpha*$_1$-receptors in mammalian neurointermediate lobe tissue.

## INTRODUCTION

The role of catecholamines in controlling pituitary function has been and is currently an important field of research (1, 2). Catecholamines have been implicated in control of the secretion of adrenocorticotropic hormone, α-melanocyte-stimulating hormone and endorphins (3–5), growth hormone (6), and prolactin (6, 7). Although most authors have concluded that norepinephrine acts at the level of the hypothalamus (3, 8), the possibility that there is a more direct mechanism remains feasible.

If norepinephrine does directly modulate pituitary function, adrenergic receptors must be present in pituitary tissue. *Beta*-adrenergic receptors have been detected in preparations from the anterior and intermediate pituitary lobes (9, 10). Agonist interaction with *beta*-adrenergic receptors stimulates cyclic AMP formation and prolactin secretion (9, 10).

The demonstration of dopamine receptors in pituitary tissue using radioreceptor binding methodology (7, 11) greatly advanced research into the role of dopamine in controlling pituitary function. We decided to use the specific *alpha*$_1$-adrenergic receptor ligand [$^3$H]prazosin to determine (a) whether any *alpha*$_1$ receptors could be detected in homogenates of anterior and/or neurointermediate lobe tissue and (b) the pharmacological properties of the *alpha*$_1$-receptors detected in order to compare these receptors with the *alpha*$_1$-receptors detected by [$^3$H]prazosin in homogenates of brain tissue.

## MATERIALS AND METHODS

Neurointermediate lobes of frozen porcine pituitaries (Boknek Biologicals, Toronto) were dissected free of anterior lobe and stalk. Homogenates (20 volumes, w/v) prepared in cold 50 mM Tris-HCl (pH 7.4 at 37°) with a glass-Teflon homogenizer were passed through cheesecloth and centrifuged (10 min, 35,000 × *g*). The pellet was resuspended in fresh buffer (40 volumes, w/v) and centrifuged once more (35,000 × *g*). Final resuspension of the pellet (25 volumes, w/v) was in 50 mM Tris-HCl (pH 7.4 at 37°). Typically, 1 g (wet weight) was equivalent to 25 mg of protein as determined by the method of Lowry *et al.* (12).

Brain tissue was prepared as described by Leysen *et al.* (13) with minor modifications. The prefrontal and parietofrontal cortices of female Wistar rats (180 g) were homogenized in 0.25 M sucrose (1:10, w/v) and centrifuged at 1,086 × *g* for 10 min. The supernatant was diluted (1:40, w/v) in 50 mM Tris-HCl buffer (pH 7.4) and centrifuged at 35,000 × *g* for 10 min. The pellet was resuspended in buffer and recentrifuged. The final suspension was in 50 mM Tris-HCl buffer (pH 7.4) at a tissue concentration of 16 mg (wet weight)/ml. At this tissue concentration, less than 5% of the total $^3$H-ligand was bound.

[$^3$H]Prazosin (28 Ci/mmole) was obtained from Amersham Corporation (Arlington Heights, Ill.). The nonradioactive drugs were obtained from various sources: piperoxan and ketanserin were generous gifts from Dr. J. Leysen of Janssen Pharmaceutica (New Brunswick, N. J.); epinephrine, norepinephrine, dopamine, yohimbine, and serotonin were obtained from Sigma Chemical Company (St. Louis, Mo.).

Assays were performed in triplicate in a 2.5-ml volume containing 4 mg (wet weight) of brain tissue or 10 mg of pituitary tissue (which was added last). [$^3$H]Prazosin saturation analyses were performed at varying concentrations of $^3$H-ligand using 1 μM phentolamine to define specific

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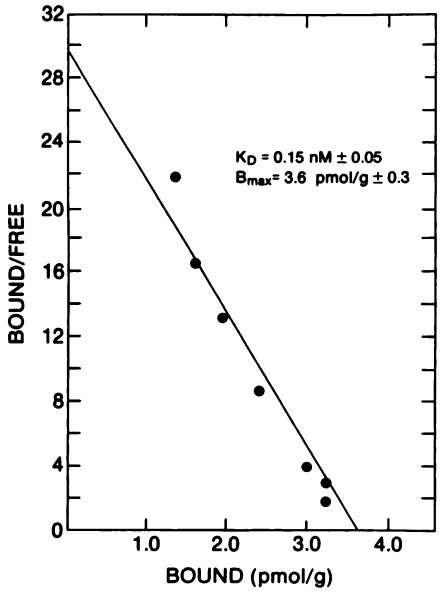


FIG. 1. *Representative Scatchard analysis of [$^3$H]prazosin specific binding to homogenates of porcine neurointermediate lobe tissue*

Phentolamine (1 μM) was used to define nonspecific binding. The points plotted are from one experiment, and each point represents determinations performed in triplicate. The mean $K_D$ and $B_{max}$ values from three experiments were 0.15 nM ± 0.05 and 3.6 pmoles/g ± 0.03, respectively. *Pmol/g* refers to amounts of receptor per gram (wet weight) of tissue. The data were analyzed according to a nonlinear regression analysis using the least-squares method. The units for bound/free are pmoles/g·nM$^{-1}$.

binding. Competition experiments were performed using 0.5 nM [$^3$H] prazosin; at this concentration, 80%–90% of total binding was specific in both tissues. Tubes were incubated for 15 min (at which time equilibrium was reached) at 37°, filtered on Flow Laboratories glass-fiber filters, and washed with 10 ml of buffer (50 mM Tris-HCl, pH 7.4). The filters were counted by liquid scintillation spectrometry in 8 ml of aqueous counting scintillant ACS (Amersham Corporation) at an efficiency of 45%. Protein concentration was determined by the method of Lowry *et al.* (12).

To ensure that no degradation of agonists was occurring, two preliminary experiments were performed. The inclusion of pargyline, a monoamine oxidase inhibitor, was found to have no effect on the results of competition experiments. Second, 10 μM norepinephrine was preincubated with membranes, and the membranes were sedimented by centrifugation; the resulting supernatant was found to be equipotent in competition experiments with a freshly prepared solution. The $EC_{50}$ and pseudo-Hill coefficient of the competition curves were essentially identical whether the assay was conducted at 15, 30, or 60 min.

## RESULTS

The specific binding of [$^3$H]prazosin to homogenates of pituitary neurointermediate lobe tissue demonstrated saturability and high affinity. Scatchard analysis (Fig. 1) indicated an apparent equilibrium dissociation constant ($K_D$) of 0.15 nM and 3.6 pmoles/g of tissue total number of sites ($B_{max}$). There was no detectable specific binding of [$^3$H]prazosin in the anterior pituitary tissue (data not shown).

In order to determine the pharmacological characteristics of the specific binding of [$^3$H]prazosin to the neurointermediate lobe, varying concentrations of adrenergic agonists and antagonists were coincubated with the $^3$H-ligand and tissue (Figs. 2 and 3; Table 1). The amounts of a series of drugs that inhibited 50% of the specific signal ($IC_{50}$) are listed in Table 1. The order of potency of antagonists, as well as their absolute potency in inhibiting [$^3$H]prazosin binding, is consistent with the pharmacological profile of an *alpha*$_1$-adrenergic receptor (Table 1; see Discussion).

In order to determine whether these pituitary *alpha*$_1$-receptors possess properties similar to those of brain, [$^3$H]prazosin binding experiments in rat brain tissue were performed in parallel with the pituitary binding studies (Table 1; Fig. 4). The correlation demonstrated in Fig. 4 indicates that the receptors in both tissues are qualitatively similar. There are some noticeable exceptions which are discussed below. The high affinity displayed by chlorpromazine for [$^3$H]prazosin binding in brain and

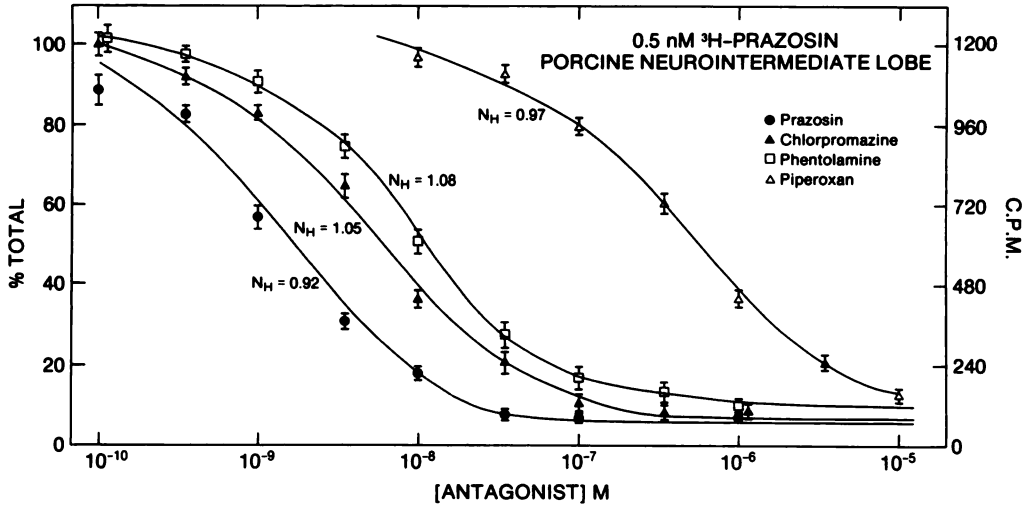


FIG. 2. *Competition by nonradioactive antagonists for the specific binding of [$^3$H]prazosin to homogenates of porcine neurointermediate lobe tissue*

Each point represents the mean of four experiments, and each point in each experiment represents determinations performed in triplicate. The error bars indicate the standard error of the mean. The range of radioligand concentrations was 0.47–0.54 nM; the tissue amount was 4 mg (wet weight).

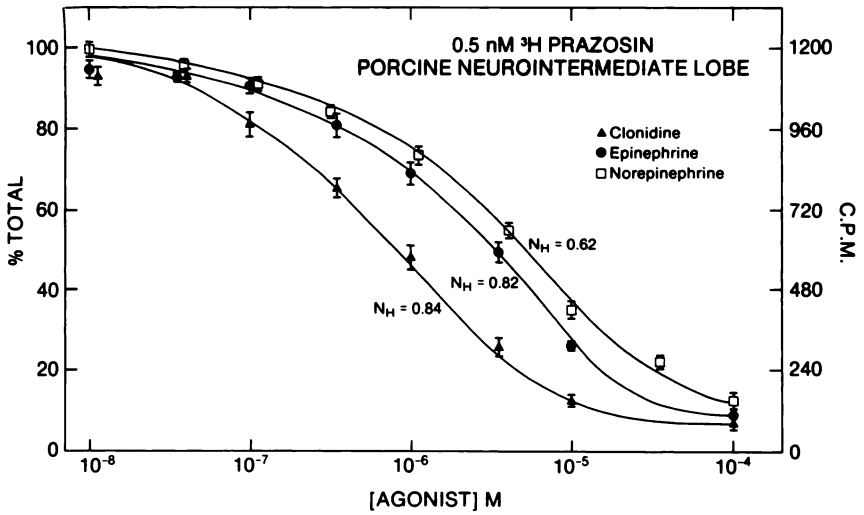


FIG. 3. *Competition by nonradioactive agonists for the specific binding of [³H]prazosin to homogenates of porcine neurointermediate lobe tissue*

Each point represents the mean of four experiments, and each point in each experiment represents determinations performed in triplicate. The error bars indicate the standard error of the mean. The range of radioligand concentrations was 0.47–0.54 nM; the tissue amount was 4 mg (wet weight).

pituitary is not unusual, as it is well documented that chlorpromazine is very potent in binding to *alpha*$_1$-receptors (14). Other calmodulin-directed agents were not investigated.

The binding site was heat-labile, as boiling the pituitary tissue for 10 min resulted in total loss of specific [³H] prazosin binding (data not shown).

TABLE 1

*Comparison of drug effects on [³H]prazosin-labeled alpha*$_1$*-receptors in rat frontal cortex and porcine neurointermediate lobe membrane homogenates*

$IC_{50}$ values for inhibition of 0.5 nM [³H]prazosin specific binding to homogenates of rat frontal cortex and porcine neurointermediate lobe tissue. Each drug was tested at eleven concentrations four times, and each is representative of experiments performed in triplicate. $IC_{50}$ values were determined by conversion of competition curve data to Hill plots. The standard error of the mean was less than 5% for all values.

| Drug | $IC_{50}$ | |
|---|---|---|
| | Rat frontal cortex | Porcine neurointermediate lobe |
| | *nM* | |
| **Antagonists** | | |
| Prazosin | 1.4 ± 0.2 | 1.0 ± 0.1 |
| Phentolamine | 50 ± 12 | 11.2 ± 2.3 |
| Phenoxybenzamine | 1.0 ± 0.2 | 7.0 ± 1.1 |
| Chloropromazine | 14 ± 2 | 4.7 ± 0.4 |
| WB-4101 | 6.4 ± 3.8 | 0.4 ± 0.1 |
| Piperoxan | 1,810 ± 260 | 341 ± 20 |
| Yohimbine | 1,330 ± 140 | 696 ± 28 |
| Clozapine | 38 ± 14 | 9.0 ± 1.0 |
| Cinanserin | 6,660 ± 520 | 3,790 ± 110 |
| Ketanserin | 52 ± 6 | 8 ± 2 |
| **Agonists** | | |
| (−)-Epinephrine | 6,730 ± 640 | 1,870 ± 80 |
| Norepinephrine | 10,700 ± 920 | 3,810 ± 640 |
| Clonidine | 1,800 ± 50 | 486 ± 72 |
| Dopamine | >100,000 | >100,000 |
| Serotonin | >100,000 | >100,000 |
| (+)-Epinephrine | 26,400 ± 1,200 | 10,200 ± 1,100 |

## DISCUSSION

[³H]Prazosin binding studies have generally demonstrated that this ³H-ligand is a high-affinity, specific ligand for *alpha*$_1$-adrenergic receptors (15, 16). The interaction of nonradioactive drugs with the [³H]prazosin sites in competition assays generally has led investigators to conclude that agonists and antagonists compete with curves of normal steepness, i.e., pseudo-Hill coefficients close to unity (15–17).

The data presented herein indicate the presence of *alpha*$_1$-adrenergic receptors in homogenates of tissue from the neurointermediate lobe. The high potency of prazosin, phentolamine, WB-4101, and phenoxybenzamine as demonstrated by competition assay for [³H] prazosin specific binding (Fig. 2; Table 1) is unique to the *alpha*$_1$-adrenergic receptor (15, 16). Epinephrine and norepinephrine are far more potent than dopamine or serotonin in inhibiting [³H]prazosin specific binding (Fig. 3; Table 1). Finally, the strong correlation between the $IC_{50}$ values determined in rat brain [³H]prazosin binding experiments and porcine pituitary experiments (Fig. 4) indicate that the *alpha*$_1$-adrenergic receptors are generally similar in the two tissues.

There are some interesting differences between the interactions of several competing drugs in the rat brain studies and the porcine neurointermediate lobe studies. The agonists epinephrine, norepinephrine, and clonidine demonstrate 3 to 4-fold higher potency at the *alpha*$_1$ sites in pituitary tissue than in brain tissue (Fig. 3; Table 1). These differences may be attributed to any of several possible causes: (a) species differences in *alpha*$_1$-receptors between hog and rat; (b) tissue differences in *alpha*$_1$-receptors between pituitary and brain; and (c) different qualities and/or amounts of modulators of *alpha*$_1$-receptors in the two preparations. This last possibility is especially intriguing as there have been no reports of a modulator of *alpha*$_1$-receptor binding until very recently (17–19). The basis of the differences in agonist affinities is currently being investigated.

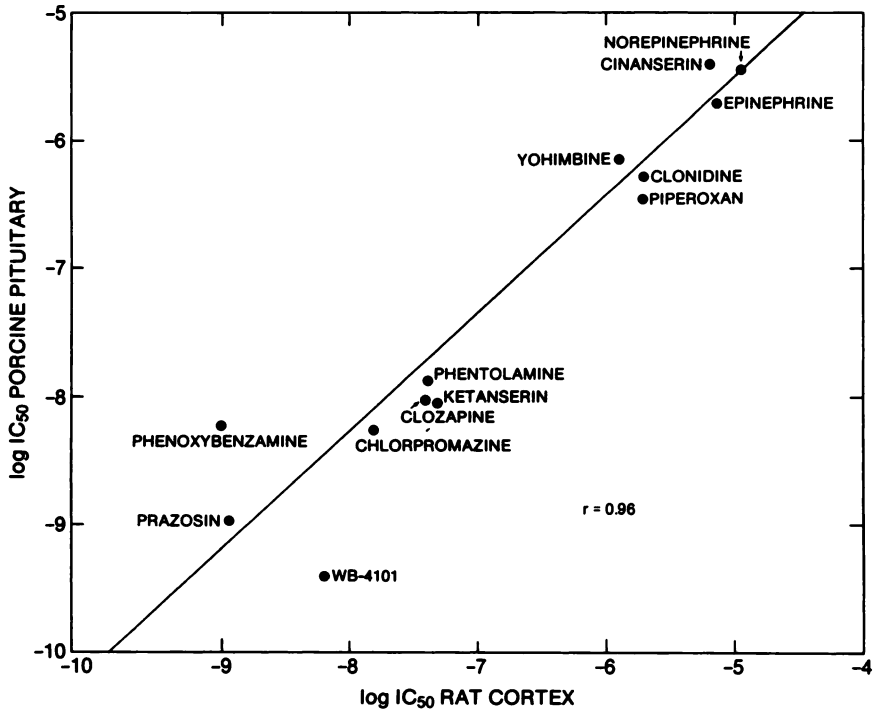


FIG. 4. *Correlation of the $IC_{50}$ values of 13 drugs determined in competition assays for [$^3$H]prazosin binding in rat frontal cortex and porcine neurointermediate lobe tissue (see Table 1)*

Antagonist potencies tend to vary randomly (Table 1). Of special interest is the 6- to 7-fold higher potency of ketanserin for *alpha*$_1$-receptors in pituitary tissue relative to brain tissue (Table 1). Although [$^3$H]ketanserin specific binding in rat brain tissue has been found to be selective for S-2 serotonin receptors (13), in tissue from the porcine pituitary neurointermediate lobe, [$^3$H]ketanserin has been found to bind to both *alpha*$_1$- and S-2 receptors.[4] These results are comprehensible if [$^3$H]ketanserin has too low a potency at *alpha*$_1$-receptors in brain to label these sites whereas in pituitary tissue [$^3$H] ketanserin has sufficiently high potency to bind significantly to *alpha*$_1$-receptors.

The results of this study represent the first demonstration of *alpha*$_1$-receptors in mammalian neurointermediate pituitary tissue. It is possible that *alpha*$_1$-adrenergic agents may regulate gonadotropin or other hormone secretions through the *alpha*$_1$-receptors in this tissue.

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