Br. J. Pharmac. (1982), **77**, 177–184

A STUDY OF α_1-ADRENOCEPTORS IN RAT RENAL CORTEX: COMPARISON OF [^{3}H]-PRAZOSIN BINDING WITH THE α_1-ADRENOCEPTOR MODULATING GLUCONEOGENESIS UNDER PHYSIOLOGICAL CONDITIONS

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1 A comparison has been made of the α_1-adrenoceptor controlling gluconeogenesis in tubules from rat renal cortex and [^{3}H]-prazosin binding in membranes prepared from the same tissue under physiological conditions.

2 In renal tubules the α-adrenoceptor agonists, oxymetazoline, (−)-noradrenaline, (−)-α-methylnoradrenaline and (−)-phenylephrine, stimulated gluconeogenesis from pyruvate. Oxymetazoline was the most potent agonist (EC_{50} 15.7 nM) but produced only 61% of the maximum response elicited by (−)-noradrenaline.

3 The α-adrenoceptor antagonists, BE2254, prazosin, indoramin and phentolamine inhibited (−)-noradrenaline-mediated increases in gluconeogenesis. The α_1-adrenoceptor selective compounds, BE2254 and prazosin, were the most effective antagonists with K_B values of 0.74 and 1.47 nM respectively.

4 [^{3}H]-prazosin binding to membranes prepared from rat renal cortex in physiological saline at 37°C was best described by a two site model. High affinity, but not low affinity sites had characteristics consistent with α-adrenoceptors.

5 High affinity [^{3}H]-prazosin binding could be completely displaced by the α-adrenoceptor agonists, oxymetazoline, (−)-noradrenaline, (−)-phenylephrine, and (−)-α-methylnoradrenaline. Slope factors for the displacement curves were all significantly less than unity. The concentrations of agonists required to displace [^{3}H]-prazosin binding were markedly higher than those required to stimulate gluconeogenesis.

6 High-affinity [^{3}H]-prazosin binding was also displaced by the α-adrenoceptor antagonists, prazosin, BE2254, phentolamine and indoramin. Slope factors for the displacement curves were close to unity. K_i values calculated from the binding experiments were very similar to K_B values obtained in the gluconeogenesis studies. These results suggest that in rat renal cortex the α_1-adrenoceptor labelled by [^{3}H]-prazosin is probably that which stimulates gluconeogenesis.

Introduction

Gluconeogenesis in the renal cortex of the rat is stimulated by catecholamines acting on α-adrenoceptors (Guder & Rupprecht, 1975; MacDonald & Saggerson, 1977). The use of selective agonists and antagonists has indicated that the receptor involved is of the α_1-subtype (Saggerson, Kessar & Carpenter, 1980; Kessar & Saggerson, 1980).

The concept that α-adrenoceptors exist as two subtypes α_1 and α_2 has been supported by the development of a wide range of selective agonists and antagonists. One of the most selective α_1-adrenoceptor antagonists, prazosin, has been shown in pharmacological (Cambridge, Davey & Massingham, 1977; Davey, 1980) and radioligand studies (U'Prichard, Charness, Robertson & Snyder, 1978; Hornung, Presek & Glossmann, 1979; Miach, Dausse, Cardot & Meyer, 1980) to be some 4 orders of magnitude more selective for α_1- than α_2-adrenoceptors. Recently, high specific activity [^{3}H]-prazosin has been used as a molecular probe for α_1-adrenoceptors in rat brain (Greengrass & Bremner, 1979; Hornung *et al.*, 1979; Summers, Jarrott & Louis, 1980), lung (Barnes, Karliner, Hamilton & Dollery, 1979), and rat kidney (McPherson & Summers, 1981; Schmitz, Graham, Sagalowsky & Pettinger, 1981). In all tissues the ligand binds with high affinity (K_D 0.1–0.6 nM) to sites having the pharmacological characteristics of α_1-adrenoceptors. In rat kidney, [^{3}H]-prazosin binding is largely confined to renal cortex (McPherson & Summers, 1981)

0007-1188/82/090177-08 $01.00

as is gluconeogenesis (Guder & Schmidt, 1974). Other parallels include the finding that in guinea-pig renal cortex, which has little gluconeogenic capacity (Stumpf & Kraus, 1977), there is negligible specific [^{3}H]-prazosin binding (McPherson & Summers, unpublished observations). In the present study the relationship between [^{3}H]-prazosin binding and the α_1-adrenoceptor-mediated increase in gluconeogenesis has been studied in preparations from rat renal cortex.

Methods

Gluconeogenesis in isolated renal tubules

Gluconeogenesis was studied in renal tubule preparations from the renal cortex of Sprague-Dawley rats (150–250 g, male or female) that had been deprived of food for 24 h (MacDonald & Saggerson, 1977). Incubations were carried out in a volume of 1 ml in 20 ml siliconized glass scintillation vials at 37°C in an atmosphere of 95% O_2:5% CO_2 in Krebs bicarbonate buffer of the following composition (mM): NaCl 119, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.18, $NaHCO_3$ 25, $CaCl_2$ 1.27 with the addition of fatty acid-free bovine serum albumin (BSA, 10 mg/ml) and with pyruvate (5 mM) as substrate. After 30 min incubation the reaction was stopped by addition of 125 µl of 45% perchloric acid, the pH adjusted to 7.4 by addition of 125 µl triethanolamine (1 M) and 125 µl potassium carbonate (5 M), centrifuged at 9,000 *g* for 4 min and an aliquot (0.5 ml) of supernatant assayed for glucose by the hexokinase method (Glucoquant-Boehringer).

Results are expressed in terms of nmol glucose produced per mg protein per hour (nmol mg^{-1} protein h^{-1}). In all cases the amount of glucose produced from other (non-pyruvate) sources was measured in controls where no substrate was added and the appropriate corrections made. Total protein concentration was measured against BSA standard in 1 ml samples of stock suspension obtained after washing 3 times with 10 ml volumes of BSA free Krebs-Bicarbonate (Lowry, Rosebrough, Farr & Randall, 1951).

The increase in rate of glucose production induced by the α-adrenoceptor agonists (−)-noradrenaline, (−)-α-methylnoradrenaline, (−)-phenylephrine and oxymetazoline was assessed by adding increasing amounts of the agonist to duplicate tubes and establishing a concentration-effect curve for each drug. The concentration producing 50% of the maximal response (EC_{50}) was then estimated graphically.

Antagonism of (−)-noradrenaline-induced increases in gluconeogenesis by prazosin, BE2254, indoramin and phentolamine was also studied. Two different concentrations of antagonist were added to duplicate tubes with increasing amounts of (−)-noradrenaline. K_B values (Furchgott, 1972) were then calculated from the shift to the right of the (−)-noradrenaline concentration-effect curve.

[^{3}H]-prazosin binding in membranes prepared from renal cortex

Renal cortex was dissected from rat kidney, weighed and homogenized (Polytron PT10, full speed, 30 s) in either 50 mM Tris/HCl, pH 7.6 (McPherson & Summers, 1981) or phosphate buffered physiological saline (PBPS, composition (mM): NaCl 119, KCl 4.8, $MgSO_4$ 1.2, NaH_2PO_4 10 and $CaCl_2$ 1.27; pH 7.4).

The characteristics of [^{3}H]-prazosin binding were studied in Tris buffer at 25°C or in PBPS at 37°C by addition of increasing amounts of [^{3}H]-prazosin (final concentration 0.01–15 nM) to tubes containing 5 mg wet weight of tissue. Non-specific binding was determined at each concentration of [^{3}H]-prazosin in parallel samples containing in addition, 10 µM phentolamine. The final volume of incubation was 2 ml and samples were incubated in a shaking water bath for 30 min. Bound and free ligand was separated by filtration through Whatman GF/B filters (selected for low filter blank) and washed with 3 × 5 ml aliquots of ice cold buffer. After air drying, [^{3}H]-prazosin retained on the filters was eluted by a toluene based scintillant (PPO 0.4%, POPOP 0.01% w/v) and counted at approximately 55% efficiency in a Searle Delta 300 Liquid Scintillation Counter.

Displacement of [^{3}H]-prazosin binding by α-adrenoceptor agonists and antagonists

The ability of α-adrenoceptor agonists and antagonists to displace [^{3}H]-prazosin binding was tested in PBPS at 37°C in a 2 ml volume. The effects of the displacing agents were studied on high affinity binding by using a [^{3}H]-prazosin concentration of 0.1 nM, and on low affinity binding by using 15 nM (1.5 nM [^{3}H]-prazosin + 13.5 nM 'cold' prazosin). At these concentrations the amount bound (B) can be estimated using the following equation:

$$B = \frac{B_{max_1}.L}{K_{D1} + L} + \frac{B_{max_2}.L}{K_{D2} + L}$$

where $B_{max_1} = B_{max}$ high affinity site (nM)
$B_{max_2} = B_{max}$ low affinity site (nM)
L = ligand concentration (nM)
K_{D1} = Dissociation constant high affinity site (nM)
K_{D2} = Dissociation constant low affinity site (nM)

It can be calculated that at the two ligand concentra-

tions chosen 95 and 73% of binding was to the high and low affinity sites respectively. Each drug was tested at 4–7 concentrations and the binding parameters estimated as indicated below.

Analysis of results

The output (ct/min) from the scintillation counter was interfaced with a Teletype 43 paper tape punch. The completed data tape was then read into a data file on the University of Melbourne VAX/VMS computer. The data were converted to d/min and processed by the computer programme 'EBDA' (McPherson 1982, in preparation). This provides estimates of equilibrium binding parameters by Scatchard and Hill Analysis using a least squares fitting procedure and also produces an output file for use in the non-linear curve fitting programme 'LIGAND' (Munson & Rodbard, 1980) which was used to obtain final parameter estimates. Both programmes are capable of handling either saturation analysis or drug displacement data.

Drugs and chemicals

The following were used: collagenase (Type II), bovine serum albumin (fatty acid-free), (−)-noradrenaline bitartrate (Sigma), pyruvate (Boehringer Mannheim), (−)-α-methylnoradrenaline, (+)-noradrenaline bitartrate (Sterling-Winthrop), (−)-phenylephrine hydrochloride (Koch-Light), oxymetazoline hydrochloride (Allen and Hanburys), prazosin hydrochloride (Pfizer), BE2254 (2(β-(4-hydroxyphenyl)ethyl-aminomethyl)-tetralone) (Beiersdorf AG), indoramin (Wyeth), phentolamine hydrochloride (Ciba), [^{3}H]-prazosin (20.2 Ci/mmol, Radiochemical Centre, Amersham). All other chemicals were 'Analar' grade.

Results

Effect of α-adrenoceptor agonists on gluconeogenesis

The α-adrenoceptor agonists, (−)-noradrenaline, (−)-α-methylnoradrenaline, (−)-phenylephrine and oxymetazoline stimulated gluconeogenesis from pyruvate in tubules from rat renal cortex in a dose-dependent manner, as shown in Figure 1. The rank order of potency of the agonists was oxymetazoline > (−)-noradrenaline > (−)-α-methylnoradrenaline > (−)-phenylephrine the EC_{50} values being 15.7 ± 1.5, 101 ± 15, 253 ± 129 and 490 ± 171 nM respectively (Table 1). The increases in rate of glucose production were for (−)-

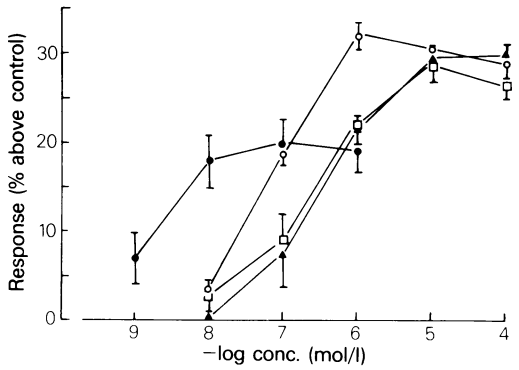

Figure 1 Effect of α-adrenoceptor agonists on gluconeogenesis from pyruvate in rat renal tubules. Percentage increases in the rate of glucose production are plotted against agonist concentration. Curves are shown for the following agonists: (−)-noradrenaline (○), (−)-phenylephrine (▲), (−)-α-methylnoradrenaline (□) and oxymetazoline (●).

Table 1 Effect of α adrenoceptor agonists on gluconeogenesis from pyruvate in rat renal tubules. Shown are the basal rate of glucose production in the absence of added agonist, the maximal increase in the presence of agonist, the % increase and the EC_{50} in nM for each agonist

Agonist	n	*Basal rate* (nmol mg^{-1} protein h^{-1})	*Maximal increase* (nmol mg^{-1} protein h^{-1})	*% increase in glucose production*	EC_{50} (nM)	*Intrinsic activity*
(−)-Noradrenaline	15	309 ± 10	408 ± 14	33 ± 1	101 ± 15	1.00
(−)-α-Methylnoradrenaline	4	322 ± 32	427 ± 46	31 ± 2	253 ± 129	0.94
(−)-Phenylephrine	4	317 ± 35	421 ± 33	35 ± 6	490 ± 171	1.06
Oxymetazoline	4	293 ± 18	355 ± 24	20 ± 3	15.7 ± 1.5	0.61

Intrinsic activity (α) is given relative to noradrenaline (= 1). The number of animals used is given by *n*.

noradrenaline 309±10 to 408±14 nmol mg^{-1} protein h^{-1} (an increase of 33±1%. $n=15$); for (−)-α-methylnoradrenaline 322±32 to 427±46 nmol mg^{-1} protein h^{-1} (an increase of 31±2%, $n=4$); for phenylephrine 317±35 to 421±33 nmol mg^{-1} protein h^{-1} (an increase of 35±6%, $n=4$); and for oxymetazoline 293±18 to 355±24 nmol mg^{-1} protein h^{-1} (an increase of 20±3%, $n=4$). Whereas (−)-noradrenaline, (−)-α-methylnoradrenaline and (−)-phenylephrine were full agonists ($\alpha=1$, 0.94 and 1.06 respectively) oxymetazoline was a partial agonist ($\alpha=0.61$) in this system ($P<0.05$). Clonidine was also tested but failed to stimulate gluconeogenesis in concentrations up to 0.1 mM although it did antagonize the response to (−)-noradrenaline (1 μM) with an IC_{50} of 1.26 μM.

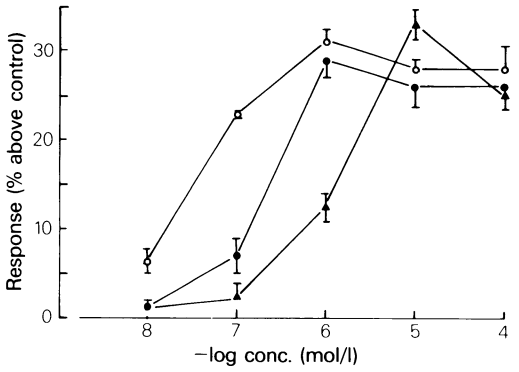

Figure 2 Effect of the α-adrenoceptor antagonist, prazosin, on stimulation of gluconeogenesis from pyruvate by (−)- noradrenaline in rat renal tubules. Percentage increases in the rate of glucose production are plotted against (−)-noradrenaline concentration in the absence (○) and in the presence of prazosin 10 nM (●) and 50 nM (▲).

Effect of α-adrenoceptor antagonists on (−)-noradrenaline-stimulated gluconeogenesis

The ability of the α-adrenoceptor antagonists, prazosin, BE2254, indoramin and phentolamine, to inhibit (−)-noradrenaline-induced increases in gluconeogenesis was studied. Prazosin (10 and 50 nM) BE2254 (10 and 50 nM), indoramin (200 and 1000 nM) and phentolamine (1 and 5 μM) all produced a shift to the right of the (−)-noradrenaline concentration-effect curve without an apparent reduction in maximum response. Mean curves for prazosin are shown in Figure 2. At the two concentrations of each antagonist used, K_B values were not significantly different (Table 2) suggesting that all are behaving in a classical competitive manner. On the basis of K_B values the rank order of potency of the antagonists was BE2254 (0.74±0.21 nM) > prazosin (1.47±0.19 nM) > indoramin (18.1±3.1 nM) > phentolamine (27.4±0.66 nM).

Characteristics of [^{3}H]-prazosin binding in membranes from rat renal cortex

In experiments where [^{3}H]-prazosin binding was studied in Tris buffer at 25°C, the binding isotherm was best described by a single site model. This was shown by the linearity of the Scatchard plots (Figure 3). In these experiments the mean K_D was 0.10±0.02 nM and B_{max} 9.96±1.40 pmol g^{-1} wet weight.

In order to make valid comparisons between parameters determined in gluconeogenesis experiments and those in binding experiments it was felt necessary to conduct both under conditions as similar as possible.

Figure 3 shows the results of experiments in which

Table 2 The effects of α-adrenoceptor antagonists on (−)-noradrenaline-stimulated gluconeogenesis from pyruvate in rat renal tubules

Antagonist	n	*Antagonist concentration* (nM)	K_B (nM)	*Mean* K_B (nM)
Prazosin	3	10	1.61± 0.33	1.47±0.19
		50	1.36± 0.23	
BE2254	3	10	0.87± 0.36	0.74±0.21
		50	0.59± 0.27	
Indoramin	3	200	19.6± 4.6	18.1±3.1
		1000	16.5± 4.9	
Phentolamine	3	1000	37.2±10.5	27.4±0.66
		5000	17.6± 3.2	

Shifts to the right of the log dose-response relationship were measured at two concentrations of antagonist. K_B values were calculated using the equation K_B = (antagonist) M/(dose ratio − 1) Furchgott (1972). The individual K_B values were used to obtain a mean K_B value.

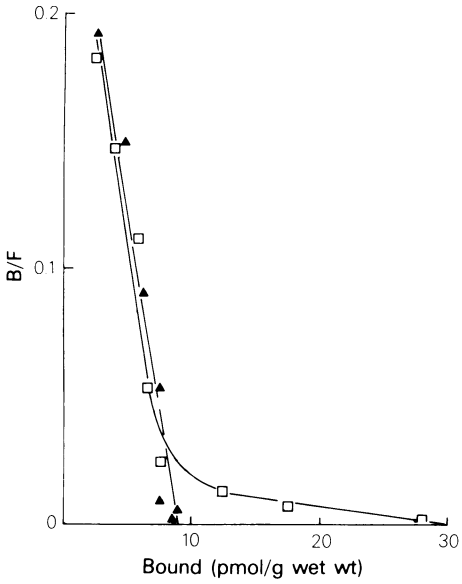

Figure 3 Scatchard plots of [^{3}H]-prazosin binding to membranes from rat renal cortex in Tris buffer at 25°C (▲) or in PBPS at 37°C (□). Using the weighted least squares fitting programme, [^{3}H]-prazosin binding in Tris buffer at 25°C was best described by a single site model. In this example the K_D was 0.052 nM and B_{max} 8.04 pmol g wet weight of tissue. [^{3}H]-prazosin binding in PBPS at 37°C was best described by a two site model, in this example, binding constants for the high affinity process were; K_D 0.043 nM and B_{max} 4.76 pmol/g wet weight of tissue. For the low affinity process the K_D was 10.8 nM and the B_{max} 23.5 pmol/g wet weight of tissue.

[^{3}H]-prazosin binding was studied in PBPS at 37°C. Under these conditions all experiments gave biphasic Scatchard plots (Figure 3) suggesting the presence of two binding sites. LIGAND analysis of this data confirmed that [^{3}H]-prazosin was binding to two sites, a high affinity site with a B_{max} of 6.2 ± 0.67 pmol^{-1} g wet weight and K_D of 0.062 ± 0.01 nM and a low affinity site with a B_{max} of 35.85 ± 7.01 pmol g^{-1} wet weight and a K_D of 17.38 ± 3.81 nM.

Displacement of high affinity [^{3}H]-prazosin binding by α-adrenoceptor agonists and antagonists

The effect of α-adrenoceptor agonists and antagonists on [^{3}H]-prazosin binding was tested in PBPS at 37°C under two conditions. At a ligand concentration of 0.1 nM, 95% of sites labelled are of the high affinity type (see Methods). Figure 4 shows the effect of the agonists oxymetazoline, (−)-noradrenaline, (−)-phenylephrine and (−)-α-methylnoradrenaline on high affinity [^{3}H]-prazosin binding. All compounds were capable of totally displacing high affinity binding. The K_i values for oxymetazoline, (−)-noradrenaline, (−)-phenylephrine and (−)-α-methylnoradrenaline were respectively 0.12 ± 0.03, 9.8 ± 1.2, 13.0 ± 2.2 and 85.1 ± 9.8 μM. [^{3}H]-prazosin binding to the high affinity site was highly stereoselective since the IC_{50} value for (+)-noradrenaline was in excess of 1 mM. In all cases the slope factors for displacement of [^{3}H]-prazosin by agonists were significantly less than unity. The results of these experiments are summarized in Table 3. Antagonists were much more potent displacers of binding than agonists and had slope factors much closer to unity. Displacement curves for prazosin, BE2254, phentolamine and indoramin are shown in Figure 4. The K_i values calculated from the IC_{50} for BE2254, prazosin, phentolamine and indoramin

Table 3 The effect of α-adrenoceptor agonists and antagonists on high affinity [^{3}H]-prazosin binding to membranes from rat renal cortex.

Agonists	IC_{50} (μM)	K_i (μM)	*Slope factor*	n
(−)-Noradrenaline	31.5 ±11.2	9.8 ±1.2	0.8 ±0.08	3
(+)-Noradrenaline	>1000	—	—	3
Oxymetazoline	0.27± 0.14	0.12±0.03	0.73±0.05	3
(−)-Phenylephrine	27.7 ± 7.3	13.0 ±2.2	0.84±0.07	3
(−)-α-Methylnor-adrenaline	123.0 ±14.0	85.1 ±9.8	0.54±0.13	4
Antagonists	IC_{50} (nM)	K_i (nM)	*Slope factor*	n
Prazosin	2.22± 1.46	1.55±1.03	0.81±0.05	4
BE2254	3.55± 1.32	0.94±0.01	0.95±0.06	5
Indoramin	155.0 ±27	70.3 ±7.25	0.89±0.05	5
Phentolamine	119.6 ±18	54.9 ±5.3	0.85±0.02	5

IC_{50} values and slope factors are obtained from iterative curve fitting of displacement curves and the K_i derived using the Cheng & Prusoff (1973) equation.

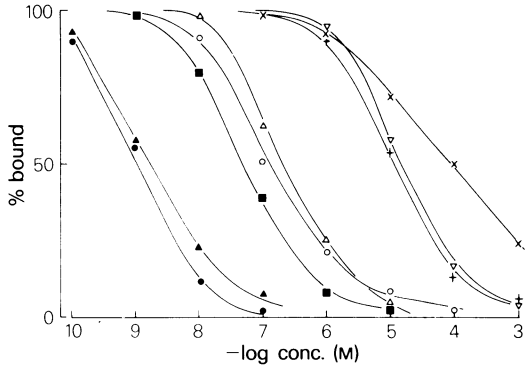

Figure 4 Displacement of high affinity [^{3}H]-prazosin binding from membranes prepared from rat renal cortex by α-adrenoceptor agonists and antagonists. Membranes were prepared as described in methods and incubated at 37°C for 30 min with 0.1 nM [^{3}H]-prazosin and 4–7 concentrations of displacer. Non specific binding was defined by 10 μM phentolamine. Typical displacement curves, determined by iterative curve fitting, are displayed for agonists, oxymetazoline (○), (−)-noradrenaline (+), phenylephrine (▽) and (−)-α-methylnoradrenaline (x) and for antagonists, BE2254 (●), prazosin (▲), phentolamine (■), and indoramin (△).

were respectively 0.94 ± 0.01, 1.55 ± 1.03, 54.9 ± 5.3 and 70.3 ± 7.25 nM. Slope factors for BE2254 and indoramin were not significantly less than unity whereas those for prazosin and phentolamine were (0.81 ± 0.05 and 0.85 ± 0.02 respectively).

Displacement of low affinity binding by α-adrenoceptor agonists and antagonists

At a ligand concentration of 15 nM the majority of sites (73%) labelled by [^{3}H]-prazosin are of the low affinity type. Low affinity binding was insensitive to catecholamines with (−)-noradrenaline, (−)-adrenaline, (−)-α-methylnoradrenaline and (−)-phenylephrine producing no measurable decrease in binding. Low affinity binding could however be displaced by antagonists and the partial agonist, oxymetazoline. The most potent displacer was prazosin with an IC_{50} of 1 μM. Oxymetazoline, phentolamine, BE2254 and indoramin were less potent with IC_{50}s in the region of 100 μM. The lack of difference in potency of these compounds indicates that the low affinity site is unlikely to be an α-adrenoceptor.

Discussion

The rate of glucose production from non-carbohydrate substrates, such as pyruvate, is elevated by α-adrenoceptor agonists (Guder & Rupprecht, 1975; MacDonald & Saggerson, 1977). Characterization of the type of receptor involved indicates that renal gluconeogenesis is stimulated through an α_1-adrenoceptor (Saggerson *et al.*, 1980; Kessar & Saggerson, 1980). The most potent agonist tested in these studies was the imidazoline, oxymetazoline (MacDonald & Saggerson, 1977). This finding has been confirmed here, since the EC_{50} for stimulation of gluconeogenesis was 15.7 nM. However, it was also shown that oxymetazoline behaved as a partial agonist in this system with an intrinsic activity of 0.61 compared to (−)-noradrenaline. Previous studies using oxymetazoline have shown it to display a spectrum of activity, from a full agonist in guinea-pig aortic strips (Wikberg, 1979) to a full antagonist in the rat mesenteric vasculature and guinea-pig splenic capsule (Hepburn & Bentley, 1980; Digges, McPherson & Summers, 1981). It is of interest that oxymetazoline should have such high potency in this system since it has been shown to display selectivity for prejunctionally located α_2-adrenoceptors (Starke, Endo & Taube, 1975; Kobinger, 1978). This may indicate that the adrenoceptors in renal tubules, although having the broad characteristics of α_1-adrenoceptors may differ in some respects from those found in other areas. The results obtained with the antagonists support the concept that the receptor is of the α_1-subtype. Comparison of K_B values for antagonists is probably a more reliable index of receptor subclassification than a comparison of the relative potency of agonists since the necessity of taking into account the efficacies of the agonists is obviated (Jenkinson, 1973; Ruffolo, Rosing & Waddell, 1979; Starke & Docherty, 1980). Thus prazosin and BE2254, which are α_1-selective (Doxey, Smith & Walker, 1977; Davey, 1980; Heinz & Hofferber, 1980) were particularly potent antagonists of noradrenaline-stimulated gluconeogenesis.

One of the primary objectives of this study was to compare the activity of α-adrenoceptor agonists and antagonists on gluconeogenesis with their effects on [^{3}H]-prazosin binding. In order for such comparisons to be valid, it was necessary to study [^{3}H]-prazosin binding under physiological conditions. It was found that under these conditions, [^{3}H]-prazosin clearly bound to two sites, a high affinity site similar to that previously observed in Tris buffer at 25°C (McPherson & Summers. 1981), and having properties of an α-adrenoceptor and a low affinity site, which did not.

It was of interest that the low affinity site was only observed when antagonists were used to define non-specific binding. When (−)-noradrenaline was used to define non-specific binding only a single population of high affinity sites with the characteristics of α-adrenoceptors was seen (McPherson & Summers, unpublished observations). It should be emphasized however that this phenomenon was only observed in kidney; in membranes prepared from rat heart and cerebral cortex under identical conditions to those used for kidney, the use of phentolamine to define

non specific binding indicated only a single high affinity site with α-adrenoceptor characteristics (McPherson & Summers, unpublished observations).

The rank order of potency for the α-adrenoceptor agonists for stimulating gluconeogenesis was oxymetazoline $\geqslant$ (−)-noradrenaline > (−)-phenylephrine > (−)-α-methylnoradrenaline and was similar to that observed for displacement of [^{3}H]-prazosin from the high affinity site. The K_i values obtained in the binding studies may not give a good guide, however, to the effectiveness of the compounds at stimulating gluconeogenesis since they give no information on the efficacy of these compounds. As expected, the correlation between the effectiveness of α-adrenoceptor antagonists in inhibiting noradrenaline-stimulated gluconeogenesis and their ability to displace [^{3}H]-prazosin from the high affinity site was more reliable. For antagonists, direct comparison can be made between K_i values obtained in binding studies and K_B values obtained against (−)-noradrenaline-stimulated gluconeogenesis. The K_i and K_B values obtained here were comparable and indicated that the α_1-adrenoceptors studied by the two methods have similar characteristics.

It has been suggested that [^{3}H]-prazosin binding in rat kidney identifies the α_1-adrenoceptor mediating renal vasoconstriction and that this ligand can be used to assess quantitatively these receptors in the rat kidney (Schmitz *et al.*, 1981). The results of the present experiments suggest that such extrapolations should be made with great care since a sizeable fraction of α_1-adrenoceptors in rat kidney appears to be linked to gluconeogenesis in renal tubules rather than blood vessels. In support of this, both [^{3}H]-prazosin binding and gluconeogenesis are present largely in renal cortex whereas blood vessels are widely distributed in both areas (Kriz, 1981). In addition, the kidney of species such as the guinea-pig where the α-adrenergic gluconeogenic response is poorly developed (Stumpf & Kraus, 1977) there is little [^{3}H]-prazosin binding (McPherson & Summers, unpublished observations).

Similarities between the α_1-adrenoceptor controlling gluconeogenesis and the high affinity site characterized by [^{3}H]-prazosin in membranes prepared from rat renal cortex can be summarized as follows: (a) [^{3}H]-prazosin binding (McPherson & Summers, 1981) and gluconeogenesis (Guder & Schmidt, 1981) are both confined to the rat renal cortex, (b) oxymetazoline is both a potent displacer of [^{3}H]-prazosin binding from the high affinity site and a potent stimulant of renal gluconeogenesis, (c) the potencies of antagonists in displacing [^{3}H]-prazosin binding from the high affinity site were similar to those obtained when assessing their ability to block (−)-noradrenaline-stimulated increases in renal gluconeogenesis and (d) in guinea-pig, where little specific [^{3}H]-prazosin binding can be detected, there is little gluconeogenesis. These findings suggest that the α_1-adrenoceptor characterized in the [^{3}H]-prazosin binding studies is probably that controlling gluconeogenesis.

Supported by the National Health and Medical Research Council of Australia of which R.J.S. is a Senior Research Fellow.

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(*Received March 31, 1982.*)