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[^{3}H]-rauwolscine binding to α_2-adrenoceptors in the mammalian kidney: apparent receptor heterogeneity between species

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1 Binding of the α_2-adrenoceptor antagonist [^{3}H]-rauwolscine was characterized in membrane preparations from the kidneys of mouse, rat, rabbit, dog, and man.

2 In all species, binding reached equilibrium within 45 min and dissociated at a single exponential rate after addition of phentolamine 10 μM.

3 Saturation studies showed that the affinity of [^{3}H]-rauwolscine was similar in all species (2.33–3.03 nM) except man where it was significantly higher (0.98 nM). Marked differences were seen in the density of binding sites, increasing in the order: man < dog < rabbit < rat < mouse. In all cases, Hill coefficients were not significantly different from unity.

4 [^{3}H]-rauwolscine binds with low affinity (K_D > 15 nM) to membranes prepared from guinea-pig kidney. The low affinity binding is not due to the absence of particular ions in the incubation medium or to receptor occupation by endogenous agonist.

5 The binding in all species was found to be stereoselective with respect to the isomers of noradrenaline. However, differences were seen in the characteristics of agonist interactions with the binding site both between isomers and between species.

6 Marked differences in affinity of particular α-adrenoceptor antagonists were observed for α_2-adrenoceptors labelled by [^{3}H]-rauwolscine. These differences were most evident with the α_1-adrenoceptor selective antagonist prazosin which displayed inhibition constants (K_i values) of 33.2, 39.5, 261, 570 and 595 nM in rat, mouse, dog, man and rabbit, respectively.

7 Differences are apparent in the characteristics of α_2-adrenoceptors labelled by [^{3}H]-rauwolscine between species and it is suggested that the differences observed for α_1-selective antagonists such as prazosin may be related to binding to additional sites in the vicinity of the α_2-adrenoceptor.

Introduction

α-Adrenoceptors have been subclassified into α_1- and α_2-subtypes based on the relative affinity of a series of agonists and antagonists. Thus an adrenoceptor is said to be of the α_1-subtype if the order of affinity of agonists is (−)-phenylephrine > clonidine > xylazine and the relative affinity of antagonists is prazosin > corynanthine > yohimbine > rauwolscine. Conversely the adrenoceptor is said to be of the α_2-subtype if this order of affinity is reversed (Wikberg, 1978; 1979; Starke, 1981).

Radioligands such as [^{3}H]-rauwolscine can be used to characterize the recognition site of the α_2-adrenoceptor provided certain guidelines are followed. It has become evident that membranes for binding studies should be prepared in hypotonic buffers to prevent vesicle formation and sequestration of endogenous neurotransmitters and modulators of binding (Cheung *et al.*, 1984). For [^{3}H]-rauwolscine binding, media should also contain ethylenediaminetetraacetic acid (EDTA) to chelate divalent cations, which alter α_2-adrenoceptor binding affinity in brain (Salama *et al.*, 1982) and it is generall held that α_2-adrenoceptor antagonist ligands are preferable to agonist ligands as the latter bind preferentially to high affinity states of the receptor (Michel *et al.*, 1980) which are markedly influenced by experimental conditions. The presence of multiple states of the α_2-adrenoceptor is

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seen as shallow competition curves for agonists competing for antagonist binding and the labelling of only a fraction of the receptor population by agonist ligands. In contrast, competition for antagonist binding by antagonists obeys simple law of mass action kinetics so that marked differences in affinity of an antagonist in competing for the binding of a ligand in preparations from different tissues or the same tissue from different species are more likely to reflect true differences in the molecular characteristics of the receptor.

Relatively few studies with antagonist ligands for α_2-adrenoceptors have been carried out in kidney. Both [^{3}H]-yohimbine (Snavely & Insel, 1982) and [^{3}H]-rauwolscine (McPherson & Summers, 1983; Tanaka *et al.*, 1983) have been used to examine α_2-adrenoceptors in rat kidney. One surprising result of these studies was the relatively high affinity of the α_1-selective antagonist prazosin for [^{3}H]-rauwolscine binding (McPherson & Summers, 1983). Similar observations have also been made in rat cerebral cortex (Cheung *et al.*, 1982; Alabaster & Brett, 1983) and rat lung (Latifpour *et al.*, 1982), guinea-pig kidney and calf cerebral cortex (Brodde *et al.*, 1983), rat submandibular gland, pig submandibular gland and pig lung (Feller & Bylund, 1984). In contrast, prazosin has much lower affinity in human tissues such as cerebral cortex and platelet (Cheung *et al.*, 1982; Summers *et al.*, 1983) and rabbit spleen (Alabaster & Brett, 1983), raising the possibility of molecular differences in the α_2-adrenoceptor between species. The object of the present study was to examine the possible heterogeneity of the renal α_2-adrenoceptor labelled by [^{3}H]-rauwolscine in a number of mammalian species, including man.

Methods

Tissue preparation

Mice (20–30 g), guinea-pigs (500–800 g) and rats (200–270 g) of either sex were killed by cervical dislocation and bled. Rabbits (2.5–3 kg) and dogs were anaesthetized with Brietal sodium (40 mg kg^{-1}, i.v.) and the kidneys perfused with 1:1 0.32 M sucrose:Krebs phosphate buffer (composition in mM; NaCl 119, KCl 4.8, $MgSO_4$ 1.2, NaH_2PO_4 10.0 and $CaCl_2$ 1.27; pH 7.6). Kidneys were removed and cleared of extraneous fat and connective tissue and placed on ice. Human kidney was obtained in one case at surgery for carcinoma of the kidney. A normal section of that kidney was used. The remaining human tissue was obtained post mortem (generally <9 h after death). No apparent difference in binding characteristics was observed between material obtained at surgery and post mortem. Tissues were generally used fresh but on occasions were stored at -70°C and used within a week.

Membrane preparations

Kidneys were homogenized in 10 vol. 50 mM Tris/HCl buffer (pH 7.4 at 4°C) for 30 s in an Ultra-Turrax homogeniser and centrifuged at 40,000 *g* for 10 min at 4°C. The supernatant was discarded and the pellet resuspended in fresh buffer and the centrifugation step repeated. The pellet was finally resuspended in 10 vol. 50 mM Tris/HCl (pH 7.4 at 25°C) containing 5 μM phenylmethylsulphonyl fluoride (PMSF), 5 mM EDTA and 0.1% ascorbate (incubation buffer). Homogenates were sieved through 210 μm nylon mesh to remove connective tissue.

Binding assay

Incubations were carried out at 25°C in a covered shaking water bath in a room with subdued filament lighting to prevent photolysis of radioligand. Binding assays were terminated by filtration through Whatman GF/B filters and washing with 3 × 5 ml aliquots of ice cold Tris buffer. Non-specific binding was determined in samples containing 10 μM phentolamine.

Kinetic experiments

The association and dissociation of [^{3}H]-rauwolscine was studied in an incubation volume of 5 ml. An equal volume of membrane suspension was added to the incubation buffer containing [^{3}H]-rauwolscine (final concentration, 1–2 nM). An equivalent mixture was incubated simultaneously but included phentolamine 10 μM to define non-specific binding. The sampling procedure consisted of vortexing the incubation mixture, taking a 200 μl sample, filtering through a GF/B filter at constant vacuum then washing with 3 × 5 ml aliquots of ice-cold buffer.

In association experiments, the homogenate was temperature equilibrated and then the mixture was sampled in duplicate at various time intervals after addition of ligand. The same procedure was used for non-specific binding estimation.

Dissociation experiments were conducted in a similar manner. The radioligand/membrane preparation was allowed to equilibrate for 45 min. The mixture was sampled before and after addition of excess phentolamine (10 μM) and dissociation followed by sampling at various times thereafter.

Saturation and drug competition experiments

Saturation and drug competition studies were conducted in 3 ml polystyrene tubes. Saturation experiments involved incubating the membranes with increasing concentrations of radioligand (0.2–24 nM). The 200 μl incubation volume consisted of equal volumes of

membrane suspension and incubation buffer containing radioligand. Non-specific binding was determined for each ligand concentration. In competition experiments, a fixed concentration of radioligand (1–2 nM) was incubated with increasing concentrations of competitor. The mixture consisted of equal volumes of membrane suspension and incubation buffer containing competitor and radioligand in a

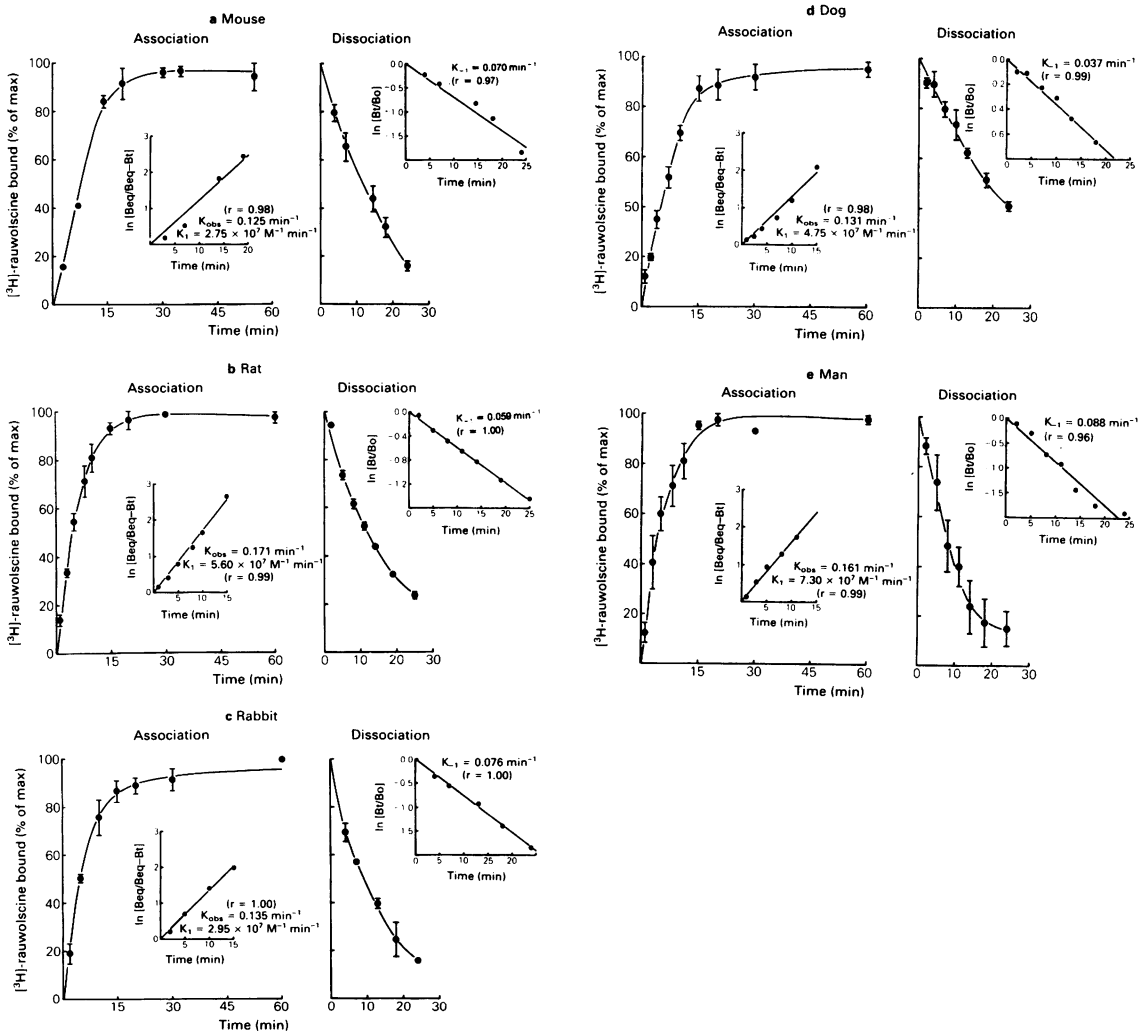

Figure 1 Kinetic analysis of [^{3}H]-rauwolscine binding to mouse (a), rat (b), rabbit (c), dog (d) and human (e) kidney membranes. Association curves represent specific binding at various times after addition of membranes to [^{3}H]-rauwolscine (1–2 nM) at 25°C. Dissociation curves represent specific binding at various times after addition of phentolamine 10 μM to membranes first incubated with [^{3}H]-rauwolscine for 45 min. Each point is expressed as a percentage of the binding occurring at equilibrium in association experiments and at time zero in dissociation experiments. Error bars indicate the s.e.mean of three separate experiments conducted in duplicate.

Association insets: second-order rate plots of [^{3}H]-rauwolscine binding. The apparent rate constant, K_{obs}, is given by the gradient of the plot of ln (Beq/Beq − Bt) against time where Beq is the specific binding at equilibrium and Bt is the binding at time t. The association rate constant, K_1, is calculated from $K_1 = (K_{obs} - K_{-1})/$[^{3}H]-rauwolscine. The dissociation rate constant, K_{-1}, was derived from dissociation experiments.

Dissociation insets: first-order rate plots of the dissociation of [^{3}H]-rauwolscine binding. K_{-1} is equal to the slope of the plot of ln (Bt/Bo) against time where Bo is the specific binding at time zero and Bt is binding at time t.

Slopes of lines were determined by linear regression analysis.

volume of 200 μl. Measurements of binding in the absence of competitor, non-specific binding and total ligand in each incubation were also taken.

Following a 45 min incubation period the reaction was terminated by the addition of 2 × 2 ml aliquots of ice-cold buffer and filtration. The filters were washed with 2 × 5 ml aliquots of buffer (filtration time < 15 s) and allowed to air-dry for 5 min.

Data analysis

Analysis of saturation and competition experiments was performed using computer-assisted iterative curve fitting (Munson & Rodbard, 1980; McPherson, 1983).

Scintillation spectrometry

Filters containing membrane-bound tritiated ligand were placed in polyethylene scintillation vials (Packard minivials). The scintillation mixture consisted of 2,5-diphenyloxazole (PPO, 0.3% w/v) and 1,4-*bis*-[2-(5-phenyloxazolyl)]-benzene (POPOP, 0.02% w/v) (Packard Instrument Company, Illinois, U.S.A.) as scintillators in 1:3 Triton X100 (Ajax Chemicals, Div. of Searle, Aust. Pty. Ltd.): xylene (May & Baker, Melbourne, Australia). Following equilibration for at least 1 h the radioactivity in the vials was counted in a Searle Delta 300 liquid scintillation counter. Corrections for counting efficiency (~40%) were made by the channels ratio method.

Drugs

Drugs used in this study were as follows: corynanthine hydrochloride, yohimbine hydrochloride, (−)-noradrenaline bitartrate, GTP (guanosine-5'-triphosphate disodium salt) (Sigma); (+)-noradrenaline bitartrate (Sterling-Winthrop); prazosin hydrochloride (Pfizer); phentolamine hydrochloride (CIBA-GEIGY); RX781094, idazoxan (Reckitt & Colman); rauwolscine hydrochloride (Roth); BE2254 (2-[β-(4-hydroxyphenyl)-ethyl-aminomethyl] tetralone) (Beiersdorf); AR-C239 bichloride (2-[2-[4(O-methoxyphenl)piperazine-1-yl] ethyl] 4,4 dimethyl-1,3,(2H-4H) isoquinolinedione Karl Thomae) (Biberach); [^{3}H]-rauwolscine sp. act. 80–85 Ci mmol^{-1} (New England Nuclear). All other chemicals were of analytical grade.

Results

Kinetics of [^{3}H]-rauwolscine binding to mammalian kidney membranes

α_2-Adrenoceptors in membranes prepared from kidneys of mouse, rat, rabbit, dog and man were characterized by use of the selective α_2-adrenoceptor antagonist [^{3}H]-rauwolscine. In all species, the binding of the ligand to the receptor was rapid and reached equilibrium within 45 min (Figure 1). The binding was reversible on addition of phentolamine (10 μM) (Figure 1) and dissociation occurred at a single exponential rate suggesting a single population of sites. Kinetically derived K_D's in mouse, rat, rabbit, dog and man were respectively, 2.55, 1.05, 2.58, 0.78 and 1.21 nM.

Saturation characteristics of [^{3}H]-rauwolscine binding

Binding was saturable as shown by the saturation isotherms of Figure 2a. Non-specific binding (defined by phentolamine 10 μM) was linear over radioligand concentrations of 0.2–24 nM (Figure 2b). Specific binding is expressed as Scatchard plots in Figure 3 for man, dog, rabbit, rat and mouse kidney membranes. Affinity of binding (− 1/gradient of plot) was similar

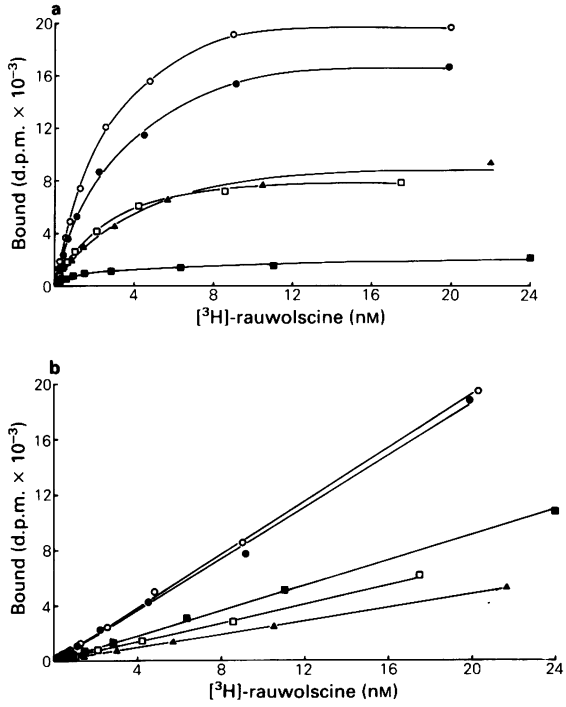

Figure 2 (a) Saturation of specific [^{3}H]-rauwolscine binding to membranes prepared from mouse (○), rat (●), rabbit (□), dog (▲) and human (■) kidney. Membranes were incubated with various concentrations of [^{3}H]-rauwolscine (0.2–24 nM) at 25°C for 45 min. Plotted curves are representative of four similar experiments conducted in duplicate. (b) Non-specific binding of [^{3}H]-rauwolscine in membranes prepared from mouse (○), rat (●), rabbit (□), dog (▲) and human (■) kidney. Membranes were incubated as in (a) but in the presence of phentolamine 10 μM.

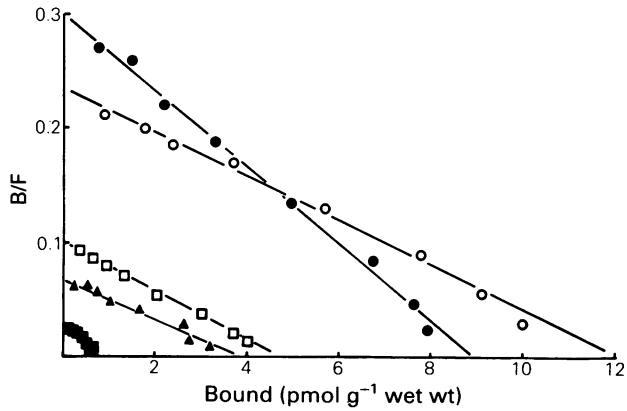

Figure 3 Scatchard plots of specific [^{3}H]-rauwolscine binding to membranes from mouse (○), rat (●), rabbit (□), dog (▲) and human (■) kidney in representative experiments. The affinity of binding (− 1/gradient of plot) was significantly higher in man (0.98 nM) than in other species (2.33–3.03 nM) ($P < 0.01$, student's t test). Differences were also seen in the density of binding sites given by the intercepts of the lines with the abscissa scale.

in mouse, rat, dog and rabbit but significantly higher in man. Marked differences were seen in the density of binding sites, increasing in the order; man < dog < rabbit < rat < mouse. Mouse kidney had more than 20 times as many [^{3}H]-rauwolscine binding sites as human kidney. Hill coefficients were not significantly different from unity, indicating that in all species [^{3}H]-rauwolscine is binding to a single population of non-interacting sites. Mean figures for all experiments are shown in Table 1.

Stereoselectivity of [^{3}H]-rauwolscine binding

Kidney membranes were labelled with [^{3}H]-rauwolscine (1–2 nM) in the presence or absence of 8 concentrations of the (−)- and (+)-isomers of noradrenaline (NA). In all species (−)-NA was more effective than (+)-NA in competing for [^{3}H]-rauwolscine binding. However, the degree of stereoselectivity varied between species, the ratio K_i(+)-NA/K_i(−)-NA increasing in the order man, rabbit, dog, rat and mouse (Table 2). As in other competition studies of agonists displacing antagonist binding, pseudo Hill coefficients were less than unity. However, two consistent trends were observed. In all cases, slope factors for (+)-NA were higher than for (−)-NA in the same species and there was variation in the slope factor between species. Thus the gradient of the competition curves for (−)-NA in human kidney was 0.54 compared to 0.83 in rabbit.

Table 1 Saturation characteristics of [^{3}H]-rauwolscine binding to mammalian kidney

Species	K_D (nM)	B_{max} (pmol g^{-1} wet wt.)	nH
Man	0.98 ± 0.02	0.73 ± 0.07	1.02 ± 0.02
Dog	3.03 ± 0.14	4.48 ± 0.29	0.98 ± 0.02
Rabbit	2.65 ± 0.27	5.45 ± 0.43	0.99 ± 0.02
Rat	2.33 ± 0.33	8.78 ± 0.35	1.00 ± 0.003
Mouse	2.79 ± 0.28	12.8 ± 0.6	0.99 ± 0.01

Figures given are mean ± s.e.mean for four experiments conducted in duplicate.

Characterization of binding by competition studies

A variety of α-adrenoceptor antagonists with different affinities and selectivity for α_1- and α_2-adrenoceptors was used in these studies. In all cases membranes were labelled with [^{3}H]-rauwolscine (1–2 nM) and 7–8 concentrations of competitor. Analysis of the competition curves for the antagonists in each species were obtained and the results are summarized in Table 3. Unlabelled rauwolscine was a potent displacer of binding with an affinity in all species ranging from

Table 2 Stereoselectivity of [^{3}H]-rauwolscine binding to mammalian kidney

Species	(−)-Noradrenaline		(+)-Noradrenaline		K_i(+)-NA / K_i(−)-NA
	K_i (μM)	− Sl.F.	K_i (μM)	− Sl.F.	
Man	1.86 ± 0.58	0.54 ± 0.04	17.2 ± 5.2	0.61 ± 0.05	9.25
Rabbit	2.69 ± 0.35	0.83 ± 0.06	32.1 ± 4.5	0.88 ± 0.02	11.9
Dog	1.45 ± 0.28	0.59 ± 0.04	28.0 ± 4.5	0.72 ± 0.06	19.3
Rat	0.74 ± 0.07	0.56 ± 0.04	18.8 ± 1.9	0.63 ± 0.06	25.4
Mouse	0.90 ± 0.10	0.66 ± 0.02	26.3 ± 2.5	0.79 ± 0.03	29.2

Slope factor values (Sl.F.) for the (+)- and (−)-isomers of noradrenaline (NA) were obtained using iterative curve-fitting of competition curves (McPherson, 1983) and the inhibition constant (K_i value) derived using the Cheng & Prusoff (1973) equation. Figures given are mean ± s.e.mean for 4 experiments conducted in duplicate.

Table 3 Competition for specific [^{3}H]-rauwolscine binding to membranes prepared from mammalian kidneys by α-adrenoceptor antagonists

Drug Competitor	*Man*		*Rabbit*		*Dog*		*Rat*		*Mouse*	
	K_i (nM)	− Sl.F.	K_i (nM)	− Sl.F.	K_i (nM)	− Sl.F.	K_i (nM)	− Sl.F.	K_i (nM)	− Sl.F.
Rauwolscine	1.70	1.03	3.34	0.98	6.21	0.92	3.22	1.09	3.37	1.06
	±0.71	±0.04	±0.21	±0.09	±0.38	±0.11	±0.31	±0.10	±0.42	±0.09
Yohimbine	2.46	0.94	14.8	0.90	14.9	0.97	9.11	1.09	10.6	1.00
	±0.71	±0.11	±2.3	±0.02	±1.0	±0.02	±0.67	±0.06	±0.7	±0.03
Idazoxan	29.6	1.02	52.5	1.01	42.5	1.18	28.5	0.97	92.5	0.96
	±2.2	±0.18	±6.8	±0.03	±2.8	±0.19	±2.7	±0.05	±7.9	±0.03
Phentolamine	35.4	0.83	64.7	1.01	41.0	0.96	39.2	1.13	47.9	0.95
	±7.0	±0.11	±8.0	±0.06	±2.3	±0.06	±2.9	±0.05	±4.6	±0.02
BE2254	8.84	1.03	27.7	0.90	10.2	1.14	9.93	1.12	12.4	0.99
	±0.95	±0.08	±4.1	±0.02	±1.0	±0.07	±2.50	±0.05	±1.2	±0.02
Corynanthine	640	1.05	706	1.04	877	1.15	475	1.02	531	1.04
	±161	±0.11	±64	±0.06	±74	±0.05	±54	±0.07	±38	±0.05
AR-C239	266	0.87	467	0.97	222	0.92	39.2	1.07	36.2	1.09
	±21	±0.04	±152	±0.14	±27	±0.05	±7.2	±0.13	±4.4	±0.08
Prazosin	570	1.09	595	0.91	261	0.94	33.2	1.06	39.5	1.10
	±36	±0.02	±70	±0.03	±33	±0.03	±4.4	±0.03	±1.9	±0.03

Inhibition constants (K_i values) and slope factors (Sl.F.) were obtained by iterative curve fitting. Data are presented as mean values ± s.e.mean for from three to nine separate determinations conducted in duplicate.

1.70–6.21 nM. While the differences shown by rauwolscine were slight, a larger degree of variation was displayed by yohimbine which was more potent in man followed by rat, mouse, rabbit and dog. Another α_2-adrenoceptor selective antagonist idazoxan (RX781094) also displayed some variation in affinity between species, being most potent in rat, followed by man, dog, rabbit and mouse. The non-selective α-adrenoceptor antagonist phentolamine showed much less variation in affinity between species with K_i values lying in the range 35.4–64.7 nM. Antagonists selective for α_1-adrenoceptors all showed marked variations in affinity for [^{3}H]-rauwolscine binding between species. BE 2254 was most effective in man followed by rat, dog, mouse and rabbit. These variations were also present with AR-C239 and prazosin but the relative order of affinities was different between species. These were for AR-C239 mouse > rat > dog > man > rabbit, and for prazosin (Figure 4) rat > mouse > dog > man > rabbit. Variations were not as marked for corynanthine. The differences could not be explained in terms of the radioligand binding to multiple sites. It has been demonstrated in the kinetic studies that the dissociation of ligand from the binding site is monophasic and in saturation studies that the Scatchard plots are linear and that Hill plots have gradients of unity. Also in the competition studies none of the slope factors of the curves differed markedly from unity (Table 3). These results indicate that the differences in K_i values are not caused by differences in slope factors in the various species due to the presence of multiple binding sites for the radioligand.

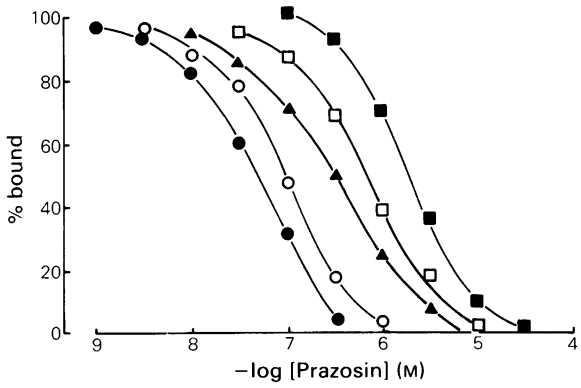

Figure 4 Comparison of prazosin competition curves in membranes prepared from mouse (○), rat (●), rabbit (□), dog (▲) and human (■) kidney. Membranes were incubated for 45 min with [^{3}H]-rauwolscine (1–2 nM) and 7–8 concentrations of prazosin. Curves showing specific binding as a percentage of control are representative of at least four experiments conducted in duplicate. Clear differences are seen in the affinity of prazosin for the site labelled by [^{3}H]-rauwolscine in different species.

[^{3}H]-rauwolscine binding in guinea-pig kidney

α_2-Adrenoceptors have been extensively characterized

and localized in guinea-pig kidney using [^{3}H]-clonidine (Jarrott & Summers, 1978; Jarrott *et al.*, 1979; Young & Kuhar, 1980). The binding of [^{3}H]-rauwolscine in this species was found to be quite unlike that in the other species studied, being of much lower affinity ($K_D > 15$ nM). A number of experiments were performed to ensure that these observations were not due to the presence or absence of particular factors. Addition of Na^+ (10 or 100 mM), Ca^{2+} (1 and 10 mM), K^+ (10 and 100 mM), Mn^{2+} (1 and 10 mM) or Mg^{2+} (1 and 10 mM), known to be modulators of binding in other systems, to the incubation medium either individually or in combination at 25°C or 37°C was found not to enhance the binding of the ligand (2 nM) by more than 10% of the control level, indicating that the low affinity binding was not due to the absence of any of these factors. To investigate whether receptor occupation by endogenous agonists was the cause of low affinity binding (Cheung *et al.*, 1984), membranes were preincubated for 30 min with either GTP (100 μM), sodium (150 mM) or both. These agents allow the agonist to dissociate from the receptor by decreasing the proportion of high affinity sites; however, no increases in binding were observed. Another approach used was to deplete the stores of noradrenaline in the kidney by treating the animal overnight (16 h) with a single intraperitoneal dose of reserpine (2 mg kg^{-1}). No increase in binding at either 25°C or 37°C in membranes prepared from the kidney of a reserpine-treated animal was observed. Therefore, on the basis of these studies, the α_2-adrenoceptor of guinea-pig kidney also appears to be distinct from those of other species since [^{3}H]-rauwolscine binds with low affinity. In spite of the low binding affinity the ligand appeared to bind to a single non-interacting population of sites since Hill coefficients in saturation studies were close to unity.

Discussion

The use of radioligands to identify the recognition sites of receptors allows the rapid and extensive characterization of these sites. During the development of the radioligand binding technique, many factors have been identified which alter the characteristics of binding. These include multiple affinity states of receptors (Tsai & Lefkowitz, 1979; Michel *et al.*, 1980; Limbird *et al.*, 1982) the proportion of high and low affinity sites being governed by monovalent cations such as Na^+, divalent cations such as Mn^{2+}, Mg^{2+} and Ca^{2+} and guanine nucleotides (Michel *et al.*, 1980). There is also recent evidence that during particular types of membrane preparation neurotransmitter can be sequestered and affect binding characteristics (Cheung *et al.*, 1984). Undoubtedly some of the differences in binding characteristics for particular binding systems are due to these factors. However, in a number of recent, carefully controlled studies differences in binding characteristics have been described in preparations from a number of tissues treated in an identical way. Comparison of [^{3}H]-rauwolscine binding to membranes from human platelets and rat cerebral cortex showed a comparatively high affinity of prazosin in the brain preparation (Cheung *et al.*, 1982). This does not appear simply to reflect a tissue difference since the characteristics of [^{3}H]-rauwolscine binding to human cerebral cortex are very similar to those in human platelets (Summers *et al.*, 1983). Recently, differences in characteristics of [^{3}H]-yohimbine binding to platelets have also been described with affinity being higher in man than rat, dog or rabbit and no specific binding demonstrable in guinea-pig platelets (Glusa & Markwardt, 1983; Kerry *et al.*, 1984). In these studies the binding characteristics correlated well with the pharmacological response of the platelets to adrenaline. In the studies described here the binding characteristics of the α_2-adrenoceptor ligand [^{3}H]-rauwolscine have been studied under carefully controlled conditions. In all species apart from guinea-pig, binding was of high affinity to a single population of sites which varied markedly in density. In the guinea-pig, binding was of much lower affinity but this effect was not due to sequestration of neurotransmitter, or to the absence of particular cations. The low affinity of [^{3}H]-rauwolscine for α_2-adrenoceptors is of interest since α_2-adrenoceptors have been successfully characterized and localized in this species using the partial agonist [^{3}H]-clonidine (Jarrott & Summers, 1978; Jarrott *et al.*, 1979; Young & Kuhar, 1980) or the rauwolscine isomer [^{3}H]-yohimbine (Brodde *et al.*, 1983)*. This could be another indication that detail differences exist between α_2-adrenoceptors in different species and that these can be identified by particular ligands.

Stereoselectivity was observed in all species but the ratio of K_i values for (+)-compared to (−)-NA varied from 9 to 29. This variation in the degree of stereoselectivity would again indicate a difference in the α_2-adrenoceptor between species. In all cases the slope factor for competition for [^{3}H]-rauwolscine was higher for (+)-NA than (−)-NA. Since the slope factor is reported to reflect the efficacy of an agonist (Kent *et al.*, 1980), this would indicate that (+)-NA always has a lower efficacy than (−)-NA at renal α_2-adrenoceptors. This lower efficacy has been observed in studies of the end organ response (Ruffolo, 1983).

Extensive characterization of the site identified by [^{3}H]-rauwolscine was made and in man, dog, rabbit, rat and mouse the ligand bound with high affinity to a

* *Note added in proof.* In our hands, [^{3}H]-yohimbine binding in guinea-pig kidney displays similar characteristics to [^{3}H]-rauwolscine binding described here.

single population of sites. This agrees with previous findings in rat kidney with [^{3}H]-yohimbine (Snavely & Insel, 1982; Pettinger *et al.*, 1982) and [^{3}H]-rauwolscine (McPherson & Summers, 1983) and is in contrast to multiple sites observed with [^{3}H]-rauwolscine in rat cerebral cortex (Diop *et al.*, 1983; Neylon & Summers, unpublished). The lower affinity of [^{3}H]-yohimbine in previous studies (Snavely & Insel, 1982; Pettinger *et al.*, 1982) can in part be attributed to the presence of Mg^{2+} ions in the incubation medium which are known to decrease antagonist binding affinity (Daiguji *et al.*, 1981). Competition curves for various antagonists gave valuable information about the characteristics of the receptors in different species. In all species tested, rauwolscine was the most potent displacer of binding; however, its affinity in man was higher than in other species. This was also seen with yohimbine and agrees with similar reports indicating that yohimbine and rauwolscine display higher affinity in human tissues such as platelet (Cheung *et al.*, 1982) and cerebral cortex (Summers *et al.*, 1983) than in rat cerebral cortex (Cheung *et al.*, 1982), submandibular gland (Feller & Bylund, 1984) and kidney (McPherson & Summers, 1983). Another α_2-adrenoceptor antagonist idazoxan (RX781094) also showed differences between species. Idazoxan has been reported to be a highly specific α_2-antagonist with a greater α_2/α_1-selectivity ratio than yohimbine in peripheral tissues (Chapleo *et al.*, 1981; Doxey *et al.*, 1983), and in central tissues using radioligand techniques (Howlett *et al.*, 1982). Some recent reports indicate that in certain tissues idazoxan may have partial agonist properties (Hannah *et al.*, 1983; Timmermans *et al.*, 1984). Changes in the apparent K_i between species could therefore be due to low slope factors caused by idazoxan acting as a partial agonist in some preparations. This does not appear to be the case here as in all situations the slope factors for competition curves were unity, indicating that idazoxan was interacting with a single population of receptors. The observed differences therefore probably represent differences in affinity of idazoxan for the site identified by [^{3}H]-rauwolscine. The non-selective antagonist phentolamine showed little difference in affinity between species displaying only a slightly lower affinity in rabbit. A similar pattern was observed for BE 2254. Although this compound is considered α_1-selective it displays only 18–31 fold selectivity for α_1-adrenoceptors some 6–7 times more selective than phentolamine (Gothert *et al.*, 1981; Adams & Jarrott, 1982). In contrast the highly selective α_1-adrenoceptor antagonists prazosin and AR-C239 showed marked variations in affinity between species with highest affinity observed in rat and mouse and the lowest in rabbit and man. The difference for prazosin was particularly marked (Figure 4).

These studies demonstrate that differences exist in the ability of α-adrenoceptor antagonists to displace [^{3}H]-rauwolscine from its binding site in renal membrane preparations from a number of species. The studies were deliberately designed using antagonists because of the problem with agonist radioligands of identification of only a fraction of the total receptor population (Michel *et al.*, 1980). In accord with this, both the radioligand and the antagonist competitors appear to identify only a single site. However, one potential problem of using antagonist radioligands is that they may not identify the precise site at which the endogenous neurotransmitter acts. It has been pointed out that agonists are small molecules relatively rich in polar groups whereas antagonists tend to be larger and composed of a hydrophobic centre and a smaller polar group (Ariens & Simonis, 1983). The characteristics of antagonists therefore tend to favour binding to lipophilic sites which are close to the polar sites acted on by the agonists. It is probably an oversimplification to think of all antagonists in these terms and another class may be relatively polar compounds with low efficacy (e.g., tolazoline) (Ruffolo, 1983). However, it is of interest that in the present study most of the selective compounds used are highly lipophilic. Attempts to examine structure-activity relationships for α_1- and α_2-adrenoceptors using models of the type suggested by McGrath (1982) must take into account several factors. Firstly, α_1- and α_2-adrenoceptors do not coexist in all tissues and where they do, they often have different concentrations and distribution. Secondly, one might expect that if the important areas for binding for yohimbine isomers are the aromatic ring, the amine substituent, and the carboxymethyl substituent (van Rossum, 1965; McGrath, 1982) one could explain differences in selectivity of drugs between species in terms of an altered spatial relationship between these areas of attachment. So for example, it has been suggested that the high selectivity of prazosin is due to its preferential attachment to the carboxymethyl subsite of α_1-adrenoceptors and that the bulk of the molecule forms an attachment to an additional site in the same plane but outside the immediate vicinity of the receptor (McGrath, 1982). A greater affinity for α_2-adrenoceptors by α_1-selective antagonists such as prazosin could be shown if the carboxymethyl site were rotated slightly in species such as the rat so allowing interaction with the α_2 site. In agreement with this model is the finding that in rat kidney where prazosin shows a high affinity for α_2-adrenoceptor binding, α_1-adrenoceptors identified by [^{3}H]-prazosin have been shown to be present (McPherson & Summers, 1981; 1982; Snavely & Insel, 1982), whereas in rat platelets where no α_1-receptors are present, prazosin has a K_i of 2000 nM (Kerry *et al.*, 1984). In addition, only a small amount of specific [^{3}H]-prazosin binding was seen in dog kidney (Neylon & Summers, unpublished observations) and little or

no binding in rabbit and human kidney. There are therefore several possible explanations for the high affinity of prazosin for the sites labelled by [^{3}H]-rauwolscine in rat kidney. The simplest is that the α_2-adrenoceptors in the various species differ in molecular characteristics. Another explanation may involve the presence of α_1-adrenoceptors on the same cells. In rat kidney, autoradiographic localization of [^{3}H]-rauwolscine and [^{3}H]-prazosin binding indicates binding predominantly to the proximal tubules (Summers, 1984; Summers *et al.*, 1984). It is possible therefore that the relatively high affinity of prazosin for the sites labelled by [^{3}H]-rauwolscine depends on the presence of α_1-adrenoceptors on the same cell membrane. However, it would be expected that these sites would saturate at low concentrations of prazosin which has very high affinity for α_1-adrenoceptors in kidney (McPherson & Summers, 1981; 1982; Snavely & Insel, 1982). A more feasible explanation may involve numerous low affinity sites (K_D 11 nM) labelled by [^{3}H]-prazosin in rat kidney (McPherson & Summers, 1982) which are increased in density in the presence of EDTA (McPherson, 1982) conditions used in the experiments described here. This large population of low affinity sites identified by prazosin in rat kidney may provide attachment points from which prazosin can compete with [^{3}H]-rauwolscine binding to α_2-adrenoceptors. The competitive nature of the interaction of prazosin with the [^{3}H]-rauwolscine binding site would indicate a close relationship between these low affinity prazosin sites and the α_2-adrenoceptor. This explanation would predict that in preparations devoid of either α_1-adrenoceptors or low affinity prazosin binding sites, the observed affinity of prazosin for [^{3}H]-rauwolscine binding is a true reflection of its affinity for the α_2-adrenoceptor. Clearly, further investigation is required to determine which of these explanations account for the apparent receptor heterogeneity.

The studies described here identify binding sites which have the appropriate molecular characteristics of α_2-adrenoceptors. However, there is increasing evidence that these sites do represent functional receptors as in rat kidney homogenates and in isolated nephron segments α_2-adrenoceptors coupled to inhibition of adenylate cyclase have been described (Woodcock & Johnston, 1982; Chabardes *et al.*, 1984). In binding studies the concentration of α_2-adrenoceptors increases in response to a high Na^+ diet in spontaneously hypertensive rats and in Dahl salt sensitive rats but not in the salt resistant strain (Pettinger *et al.*, 1982).

In operational terms the differences in receptor characteristics could be very important. Clearly the use of rat tissues to predict the relative potency of α-adrenoceptor antagonists based on binding studies could give a distorted picture of the affinity and selectivity of these compounds in man or other animal species. The present studies indicate that rabbit kidney preparations display similar affinity for antagonists to human tissues and may therefore represent a more appropriate model for human α_2-adrenoceptors. It also remains to be established that these differences are reflected in an appropriate end organ response. It also raises the exciting possibility that receptors as defined by antagonists may differ sufficiently between tissues to allow the development of compounds which can be targeted to particular areas or organs.

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