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BINDING OF [^{3}H]SCH23390 TO A NON-DOPAMINERGIC SITE IN BOVINE KIDNEY

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Abstract—Binding of the D_1 dopamine receptor antagonist [^{3}H]SCH23390 to bovine renal cortical membranes has been studied. Specific binding of [^{3}H]SCH23390 was saturable and reversible and stereoisomers of SCH23390 competed stereoselectively. In contrast, competition with the isomers of butaclamol was not stereoselective and dopamine failed to compete for the [^{3}H]SCH23390 binding site. The site is therefore not a D_1 dopamine receptor. Competition studies with a very wide range of compounds failed to define the nature of the [^{3}H]SCH23390 binding sites in renal cortex whereas in parallel studies the characteristics of [^{3}H]SCH23390 binding in caudate nucleus were entirely consistent with those of D_1 dopamine receptors. The nature of [^{3}H]SCH23390 binding in preparations of tubular and glomerular membranes was found to be virtually identical to those of crude renal cortical membranes indicating lack of compartmentation of these sites. Autoradiographic studies of [^{3}H]SCH23390 binding in bovine kidney showed significantly higher levels of binding sites in renal cortex compared with renal medulla and this was confirmed by direct ligand binding studies.

The D_1 dopamine receptor is an important pharmacologically distinct site of action of dopamine found in the brain and periphery [1, 2]. In the brain, D_1 dopamine receptors were first identified from the stimulation of adenylyl cyclase and subsequently they have been shown to play an important role in the regulation of certain behaviours by dopamine [3]. An important advance in the molecular study of D_1 dopamine receptors was the identification of benzazepine drugs e.g. SCH23390 as selective D_1 dopamine receptor antagonists and the subsequent use of [^{3}H]SCH23390 in ligand binding assays [1].

In the periphery, a number of D_1 dopamine receptor-mediated actions of dopamine have been identified. In the kidney, infusion of dopamine *in vivo* results in an increase in renal blood flow [4] and the effects of dopamine are abolished by SCH23390 [5–7]. *In vitro* studies on precontracted human renal arteries have also identified the D_1 dopamine receptor in the vasodilation caused by dopamine agonists [8]. Stimulation of adenylyl cyclase by dopamine agonists has also been reported in rat renal artery [9], renal tubular membranes [10] and cultured mesangial cells [11].

Radioligand binding studies with benzazepine antagonists have been used to identify D_1 dopamine receptors in human and rat renal cortex but the results show significant variability [12–16]. In the present paper we have carried out a detailed study comparing the binding of [^{3}H]SCH23390 to membrane preparations of bovine renal cortex and caudate nucleus in order to ascertain whether central and peripheral D_1 dopamine receptors have similar pharmacological properties.

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MATERIALS AND METHODS

Materials. [^{3}H]SCH23390 (65–75 Ci/mmol) was obtained from Amersham International, Amersham, U.K.). We acknowledge generous gifts of the following substances: piflutixol (Lundbeck, Denmark), mianserin (Beecham, Harlow, U.K.), haloperidol (Janssen Pharmaceutica, Beerse, Belgium), prazosin (Pfizer Central Research, Sandwich, U.K.) and rauwolscine (Roth, Karlsruhe, Germany). All other chemicals were of the highest purity available and were obtained from commercial sources.

Preparation of membranes. Bovine kidneys and brains were obtained from a local abattoir and transported to the laboratory on ice. Strips of renal cortex or renal medulla were dissected from the kidneys, weighed and minced with a razor blade at 4°. The tissue was then homogenized, in ice-cold 0.32 M sucrose solution (Buffer A 10 mL/g wet weight of tissue) containing 20 mM HEPES pH 7.4 and 0.1 mM phenyl methane sulphonyl fluoride, using a Potter Teflon–glass homogenizer (12–15 strokes, 850 rpm). The resulting homogenate was centrifuged (10 min, 1500 *g*), the pellet discarded and the supernatant centrifuged for 60 min at 45,000 *g*. The supernatant was then discarded and the pellet of mixed mitochondrial/microsomal membranes was homogenized (7–8 strokes, 850 rpm) in 2 mL/g wet weight of tissue of HEPES Buffer B (20 mM HEPES, 1 mM EDTA, 1 mM EGTA pH 7.4) containing a mixture of protease inhibitors [0.05% (w/v) leupeptin, chymostatin, aprotinin, pepstatin A and antipain]. The membrane preparation was stored in aliquots at −80°.

Glomeruli and tubules were prepared from renal cortex by the sieving method of Sochor *et al.* [17]. The cortical material was minced in ice-cold phosphate-

buffered saline, pH 7.4, and forced through a 500 μm mesh to disrupt the tissue. The material was then passed through a 250 μm nylon mesh on which tubules were retained and glomeruli were subsequently retained on a 125 μm nylon mesh. Light microscopic examination revealed greater than 90% purity of the tubular and glomerular fractions. Membranes were then prepared from these fractions by a similar procedure to that for renal cortex.

Bovine caudate nucleus membranes were prepared as described in Leonard *et al.* [18] by a method similar to that described for renal cortex. The mixed mitochondrial/microsomal membranes were resuspended in HEPES Buffer B with protease inhibitors as above and the preparation was stored in aliquots at −80°. Protein concentration was determined by the method of Lowry *et al.* [19] using bovine serum albumin as a standard and employing a precipitation step with trichloroethanoic acid (10%).

[^{3}H]*SCH*23390 *binding assays*. Membranes were diluted to 0.3 mg protein/mL in HEPES Buffer C (20 mM HEPES, 1 mM EDTA, 0.1% ascorbate, 120 mM NaCl, pH 7.4). Diluted membranes (0.3 mL) were incubated with [^{3}H]SCH23390 (kidney: 1–2 nM; caudate nucleus: 0.5 nM) and competing ligands in a final volume of 0.45 mL for 60 min at 22°. Non-specific binding to renal cortical membranes was defined with 1 μM non-radioactive SCH23390, and 10 μM (+)-butaclamol defined non-specific binding to caudate nucleus membranes. Non-specific binding accounted for less than 20% of total [^{3}H]-SCH23390 binding to renal cortical membranes and less than 10% of the binding to caudate nucleus membranes. The incubation was stopped by rapid vacuum filtration either through Whatman GF/B filters using a Brandel cell harvester or through Whatman 934AH filters using a Dynatech Automash 2000 cell harvester. Filters were washed with 12 mL of ice-cold phosphate-buffered saline, pH 7.4, before radioactivity trapped on the filters was determined by liquid scintillation counting.

Using the method of Golds *et al.* [20], it was shown that there was no significant metabolism of [^{3}H]SCH23390 during the ligand-binding assays.

Data analysis. Saturation binding and competition data were analysed by non-linear least squares curve fitting using the programs EBDA and LIGAND (Elsevier Biosoft) [21, 22].

Ligand autoradiography. Bovine kidney and striatal tissue were dissected and frozen immediately in isopentane/liquid nitrogen (−60°). Sections (20 μm-thick) were cut on a Leitz cryostat at −20° and thaw-mounted onto glass slides subbed with gelatin and chrome alum. The sections were stored at −20° for up to 1–2 weeks before use. The sections were incubated with [^{3}H]SCH23390 (kidney: 1 nM; striatum: 0.5 nM) in HEPES buffer C for 60 min at 22°. Non-specific binding was defined by 1 μM SCH23390 for kidney and 10 μM (+)-butaclamol for striatal sections. The slides were washed twice by immersion for 5 min in fresh buffer followed by four 10 sec washes in deionized water. Slides were dried under a stream of air and were placed against Amersham Hyperfilm-^{3}H for 2–5 weeks. Autoradiographic density was assessed by comparison with a ^{3}H-microscale (Amersham) and image analysis performed using an Amersham RAS 1000 analyser.

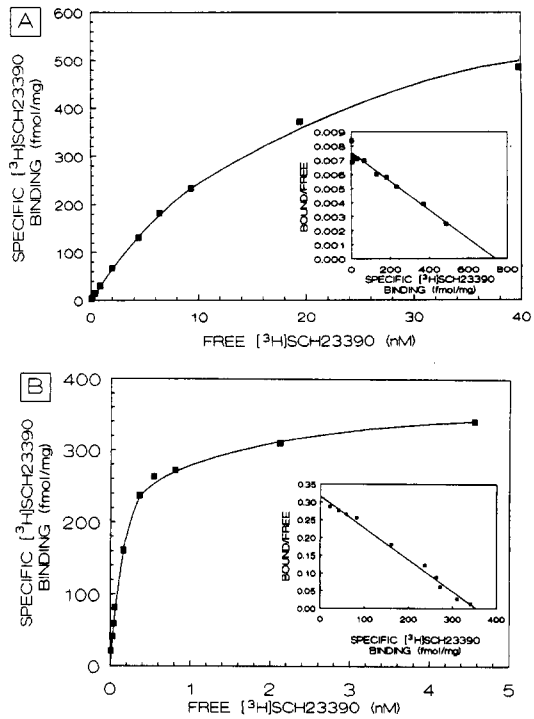


Fig. 1. Representative saturation isotherms showing [^{3}H]-SCH23390 binding to (A) renal cortical membranes and (B) caudate nucleus membranes. Renal cortical and caudate nucleus membranes were incubated with increasing concentrations of [^{3}H]SCH23390 (renal cortex: 0.1–40 nM; caudate nucleus: 0.01–5 nM) as described in Materials and Methods. Each point is the mean of triplicate determinations in a single representative experiment replicated as described in the text. Inset: representative Scatchard plots.

RESULTS

[^{3}H]*SCH*23390 *binding to bovine renal cortex and caudate nucleus membranes*

In preliminary experiments [^{3}H]SCH23390 binding to renal cortex membranes was observed. Competition with 10 μM (+)-butaclamol, the concentration used to define specific [^{3}H]SCH23390 binding in caudate nucleus showed inhibition of [^{3}H]-SCH23390 binding but similar inhibition was seen with 10 μM (−)-butaclamol. Owing to this non-stereoselectivity of butaclamol competition it was therefore decided to use 1 μM non-radioactive SCH23390 for defining specific [^{3}H]SCH23390 binding in renal cortex.

Saturation analysis of specific [^{3}H]SCH23390 binding to bovine renal cortical membranes revealed a single saturable class of binding sites with a dissociation constant (K_D) of 15.4 ± 9.4 nM and a maximal binding capacity (B_{max}) of 665 ± 129 fmol/mg protein (mean ± SD, seven experiments). A representative saturation curve is shown in Fig. 1. In bovine caudate nucleus membranes, specific [^{3}H]SCH23390 binding was saturable and to a single class of binding sites with a K_D of 0.233 ± 0.045 nM and B_{max} of 364 ± 47 fmol/mg protein (mean ± SD, seven experiments) (Fig. 1). For both renal cortex

Table 1. Inhibition of [3H]SCH23390 binding to renal cortical and caudate nucleus membranes by dopamine and serotonin receptor antagonists and agonists

Compound	Selectivity	Caudate nucleus		Renal cortex	
		K_i(nM)	Hill coefficient	K_i(nM)	Hill coefficient
SCH23390	D_1 antagonist	0.211 ± 0.036	1.15 ± 0.20	16.6 ± 6.1	1.07 ± 0.14
SCH23388	Inactive	185 ± 111	1.15 ± 0.02	1670 ± 460	1.15 ± 0.15
SKF83566	D_1 antagonist	0.452 ± 0.129	0.97 ± 0.05	32.4 ± 11.3	1.03 ± 0.04
(+)-Butaclamol	D_1/D_2	10.2 ± 3.4	1.20 ± 0.12	2030 ± 100	1.31 ± 0.46
(−)-Butaclamol	Inactive	6240 ± 1090	2.54 ± 0.16	1070 ± 30	1.34 ± 0.35
Piflutixol	$D_1 > D_2$	4.86 ± 1.88	1.52 ± 0.21	665 ± 217	1.97 ± 0.45
Fluphenazine	D_1/D_2	48.7 ± 4.4	1.34 ± 0.08	144 ± 23	1.07 ± 0.06
Trifluoperazine	D_1/D_2	81.5 ± 17.0	1.40 ± 0.19	198 ± 10	1.13 ± 0.12
(+)-SKF82526	D_1 agonist	3.36 ± 0.57	0.85 ± 0.04	6980 ± 430	1.04 ± 0.03
(−)-SKF82526	Inactive	727 ± 285	0.97 ± 0.04	16,400 ± 9600	0.86 ± 0.11
(+)-SKF38393	D_1 agonist	36.1 ± 3.0	0.96 ± 0.03	1000 ± 150	0.96 ± 0.10
(−)-SKF38393	Inactive	11,500 ± 1700	0.98 ± 0.02	41,800 ± 12,200	1.05 ± 0.31
Dopamine	D_1/D_2 agonist	364 ± 125*	0.75 ± 0.07	> 1,000,000	—
Spiperone	$D_2/5HT_2/5HT_{1a}$	627 ± 142	1.41 ± 0.46	962 ± 392	1.01 ± 0.16
Pimozide	D_2	1690 ± 170	2.18 ± 0.37	188 ± 41	1.38 ± 0.15
Haloperidol	D_2	119 ± 25	1.15 ± 0.17	1690 ± 60	1.21 ± 0.17
Mianserin	$5HT_2/5HT_{1c}$	231 ± 29	0.95 ± 0.17	13,300 ± 4800	1.41 ± 0.36
GR38032	$5HT_3$	>10,0000	—	4460 ± 890	0.96 ± 0.03
Serotonin (5-HT)	5HT	28,600 ± 2600	1.01 ± 0.09	> 1,000,000	—

Each compound was tested for inhibition of [3H]SCH23390 binding to renal cortical and caudate nucleus membranes as described in Materials and Methods. Competition experiments with dopamine, serotonin and D_1 agonists were carried out in the presence of 5 mM $MgCl_2$ and 10 μM pargyline and absence of NaCl. Values are the means of three or four experiments ± SD.

* Fits better to a two site model: $K_H = 60.5$ nM; $K_L = 0.88$ μM; % $R_L = 65.8\%$.

and caudate nucleus specific binding was completely reversible when an excess of a competing ligand was added.

In order to investigate the pharmacological profile of [3H]SCH23390 binding to these tissues, competition experiments were carried out with a range of structurally different compounds. The results for competition of [3H]SCH23390 binding with dopaminergic and serotonergic compounds are shown in Table 1. SCH23390 was 1000 times more potent than the inactive enantiomer SCH23388 in caudate nucleus membranes and 100 times more potent in renal cortical membranes, indicating the stereoselectivity of SCH23390 binding in both tissues (Fig. 2). However, although the mixed D_1/D_2 receptor antagonist (+)-butaclamol was approximately 1000 times as potent as (−)-butaclamol in caudate nucleus, the (−) enantiomer had a slightly higher affinity than the (+) enantiomer for the [3H]SCH23390 binding to renal cortex. The most striking indication that the [3H]SCH23390 binding to renal cortical membranes is not dopaminergic was the lack of competition of dopamine for [3H]-SCH23390 binding even at very high levels of the agonist (1 mM). In contrast, dopamine competed well for [3H]SCH23390 binding to caudate nucleus membranes and the data fitted better to a model of two classes of binding site. The D_1/D_2 receptor phenothiazine antagonists fluphenazine and trifluoperazine had a greater affinity than the thioxanthene, piflutixol for the [3H]SCH23390 binding site in renal cortex and this is a reversal of the potencies of these compounds for the D_1 dopamine receptor in caudate nucleus. In addition, although the agonist SKF82526 was 10-fold more potent than SKF38393 at the D_1 dopamine receptor in caudate nucleus, it had a lower affinity than SKF38393 for the renal cortex site. The stereoselectivity of the enantiomers of these compounds in competing for [3H]SCH23390 binding was also greatly reduced in the renal cortex. Hill coefficients for binding of these dopaminergic compounds to renal cortical membranes were close to 1, indicating binding to a single class of sites.

As the binding of SCH23390 to several classes of serotonin (5-HT, 5-hydroxytryptamine) receptors has been reported [23–25], competition of various serotonergic compounds for [3H]SCH23390 binding was investigated. Serotonin (5-HT), and serotonergic antagonists, exhibited rather low affinities for the [3H]SCH23390 binding site in renal cortex and so binding to a serotonergic site was excluded.

Table 2 shows the inhibition constants derived from competition experiments using a variety of compounds. Prazosin, rauwolscine and propranolol exhibited low affinities for [3H]SCH23390 binding to renal cortical membranes thus excluding the possibility of [3H]SCH23390 binding to a class of adrenergic receptors. As stated earlier, the phenothiazines fluphenazine and trifluoperazine were found to have moderate affinity for the [3H]-SCH23390 binding site in renal cortex. Binding of phenothiazines to a variety of proteins, including calcium channels [26] and calmodulin [27], and effects of phenothiazines on the cellular levels of peripheral benzodiazepine receptors [28], have been reported. Compounds specific for both peripheral and central benzodiazepine receptors were tested

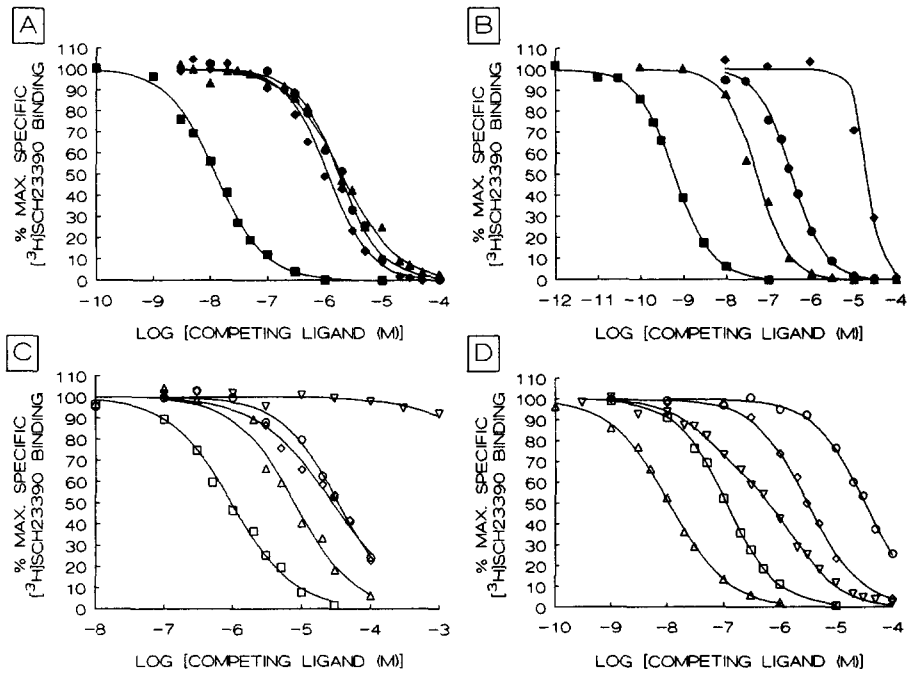


Fig. 2. Competition by dopaminergic agonists and antagonists for [^{3}H]SCH23390 binding to renal cortical and caudate nucleus membranes. Shown are representative competition curves for antagonists in renal cortex (A) and caudate nucleus (B) and for agonists in renal cortex (C) and caudate nucleus (D). Antagonist ligands in (A) and (B) were SCH23390 (■), SCH23388 (●), (+)-butaclamol (▲) and (−)-butaclamol (◆). Agonist ligands in (C) and (D) were (+)-SKF38393 (□), (−)-SKF38393 (○), (+)-SKF82526 (△), (−)-SKF82526 (◇) and dopamine (▽). The data are from representative experiments with data points in triplicate replicated as in Table 1.

Table 2. Inhibition of [^{3}H]SCH23390 binding to renal cortical membranes by various competitors

Compound	Selectivity	K_i (nM)	Hill coefficient
Prazosin	α_1 Adrenergic	13,400 ± 1500	1.10 ± 0.05
Rauwolscine	α_2 Adrenergic	13,500 ± 900	0.79 ± 0.05
Propranolol	β adrenergic	32,400 ± 12,300	0.91 ± 0.14
Ro5-4864	Peripheral BDZ	1540 ± 280	1.02 ± 0.22
Diazepam	Peripheral/central BDZ	6730 ± 2040	0.92 ± 0.08
Clonazepam	Central BDZ	6320 ± 1040	1.13 ± 0.12
Ro15-1788	Central BDZ	>100,000	—
Verapamil	Ca^{2+} channel	544 ± 61	1.00 ± 0.03
Diltiazem	Ca^{2+} channel	2830 ± 480	0.95 ± 0.06
Nifedipine	Ca^{2+} channel	14,200 ± 2600	1.00 ± 0.05
W7	Calmodulin	6780 ± 2100	1.18 ± 0.17
Histamine		>1,000,000	—
Adenosine		>1,000,000	—
Acetylcholine		>1,000,000	—
GABA		>1,000,000	—
Angiotensin II		>100,000	—
Bradykinin		>100,000	—
Vasopressin		>100,000	—

Each compound was tested for inhibition of [^{3}H]SCH23390 binding to renal cortical membranes as described in Materials and Methods. Values are the means of three to four experiments ± SD.

Each compound tested at a concentration of 10^{-4} M inhibited less than 50% of [^{3}H]-SCH23390 binding to caudate nucleus membranes, with the exception of verapamil (approx. IC_{50} = 6 μM).

BDZ, benzodiazepine receptor.

for competition of [3H]SCH23390 binding to renal cortical membranes but the pattern of affinities (Ro5-4864 > Clonazepam = Diazepam ≫ Ro15-1788) was not consistent with binding to a peripheral benzodiazepine receptor (Ro5-4864 > Diazepam ≫ Clonazepam = Ro15-1788) [29]. Similarly, the affinities of compounds representative of three classes of calcium channel antagonists (verapamil, diltiazem and nifedipine) for the [3H]SCH23390 binding site of renal cortex were not sufficiently high to suggest that binding was to a calcium channel. The calmodulin antagonist W7 also exhibited a low affinity for [3H]SCH23390 binding to renal cortex and in addition heat treatment of the membrane preparation to 75°–80° for 15 min prior to measurement of [3H]SCH23390 binding resulted in a marked decrease (60–90%) in the specific binding, whereas calmodulin is a heat stable protein [30]. The endogenous ligands histamine, adenosine, acetylcholine and γ-aminobutyric acid (GABA), as well as the endogenous peptides angiotensin II, bradykinin and vasopressin which are involved in the control of fluid balance in the kidney, were tested for inhibition of [3H]SCH23390 binding to renal cortical membranes but none of these compounds at concentrations of 10^{-4} or 10^{-3} M competed for [3H]SCH23390 binding. The possibility that the binding sites represented cytochrome P450 II D1 was investigated in preliminary competition experiments versus [3H]-SCH23390. K_i values of 2.5 μM (SKF525A), 11.4 μM (quinidine), 5.0 μM (lobeline) and >100 μM (methylphenidate) were obtained with compounds selective for this enzyme indicating that the sites were not this cytochrome. Finally, the possibility that [3H]SCH23390 binding in the renal cortex was not to a protein but was to another cellular component such as lipid was excluded by the fact that trypsin treatment of the membranes (1 mg trypsin: 20 mg membrane protein) reduced the specific [3H]SCH23390 binding to 6% of the value for membranes not treated with trypsin.

[3H]*SCH*23390 *binding to renal tubular and glomerular membranes*

The characteristics of [3H]SCH23390 binding to preparations of tubular and glomerular membranes from renal cortex (Table 3) were very similar to those of [3H]SCH23390 binding to preparations of renal cortical membranes. No significant differences between binding to tubular and glomerular membranes, in terms of the K_D and B_{max} of [3H]-SCH23390 binding and the potencies of inhibition of various dopaminergic compounds, were observed.

Autoradiographic investigation of [3H]*SCH*23390 *binding to sections of bovine kidney and bovine striatum*

[3H]SCH23390 binding to a section through bovine basal ganglia was determined autoradiographically and can be seen to be localized to the regions of the caudate nucleus and putamen, and in a section through a lobule of bovine kidney, higher levels of [3H]SCH23390 binding are observed in the cortex than in the medulla (Fig. 3). Image analysis of the autoradiographic film allowed quantitation of [3H]-SCH23390 binding to the sections by comparison of the density of autoradiographic grains over the sections with a 3H-microscale (Amersham). Specific binding of 1 nM [3H]SCH23390 was found to be 49.7 fmol/mg tissue protein to renal cortex and 3.0 fmol/mg to renal medulla which is 6.0% of the binding to renal cortex. Direct ligand binding studies of 5 nM [3H]SCH23390 to membrane preparations of these tissues showed specific binding of 163 ± 22 fmol/mg protein for renal cortex and 23 ± 4 fmol/mg for renal medulla, which is 13.8% of the binding to renal cortex.

DISCUSSION

This study shows the presence of [3H]SCH23390 binding sites in renal cortex which exhibit different pharmacological characteristics to [3H]SCH23390 binding to D_1 dopamine receptors in brain (caudate nucleus). Although [3H]SCH23390 binding is saturable and stereospecific in both the renal cortex and caudate nucleus, the affinity of [3H]SCH23390 is almost 100-fold lower for the site in renal cortex. Competition binding studies on bovine caudate nucleus membranes confirmed that in this study [3H]SCH23390 binding was to a D_1 dopamine receptor comparable to that observed by other research groups [1, 2]. However, competition binding on bovine renal cortical membranes showed a completely different pattern of inhibition of [3H]-SCH23390 binding by a range of dopaminergic compounds. The lack of competition of [3H]-SCH23390 binding by dopamine at millimolar concentrations and the lack of stereospecificity of butaclamol binding indicate that the [3H]SCH23390 binding in renal cortex is not to a functional D_1 dopamine receptor. In this study, the D_1 dopamine receptor agonist SKF38393 had a higher affinity for the renal cortex binding site than the agonist SKF82526, which is the opposite of the pattern of affinities of these compounds in caudate nucleus.

SCH23390 has been reported to bind to $5HT_2$ serotonin receptors in frontal cortex [23], serotonin $5HT_{1c}$ receptors in choroid plexus [24] and serotonin $5HT_{1e}$ receptors in platelets [25] but competition experiments with serotonergic ligands in the present study showed that [3H]SCH23390 binding to renal cortex was not to serotonergic binding sites. Similarly, competition studies with ligands specific for benzodiazepine receptors, calcium channels and calmodulin all gave affinities for these compounds in the micromolar range excluding these as target sites.

A range of endogenous compounds, such as acetylcholine, GABA, histamine, adenosine and peptides, involved in diuresis showed no inhibition of [3H]SCH23390 binding to either renal cortex or caudate nucleus. "Acceptor sites" for particular chemical components of drugs such as the spirodecanone site of [3H]spiperone binding [31] have been observed. However, the range of compounds which compete for [3H]SCH23390 binding to renal cortex vary greatly in structure from the benzazepine SCH23390, which excludes the possibility of binding to a benzazepine "acceptor site". The fact that it is only exogenous drugs and not endogenous compounds which inhibit [3H]SCH23390 binding in

Table 3. [^{3}H]SCH23390 binding to preparations of renal tubular and glomerular membranes

	Tubules		Glomeruli	
(a) Saturation analysis				
K_D (nM)	16.6 ± 11.0		14.3 ± 13.3	
B_{max} (fmol/mg)	425 ± 179		514 ± 375	
	K_i (nM)	Hill coefficient	K_i (nM)	Hill coefficient
(b) Inhibition constants from competition studies				
Compound				
SCH23390	18.8 ± 15.7	0.88	13.2 ± 6.0	0.88
SCH23388	2140 ± 1780	1.12	1390 ± 1390	0.92
(+)-Butaclamol	6730 ± 1870	1.34	5360 ± 20	1.12
(−)-Butaclamol	3680 ± 1150	1.12	3850 ± 1080	1.34
Dopamine	$>10^{-3}$ M	—	$>10^{-3}$ M	—

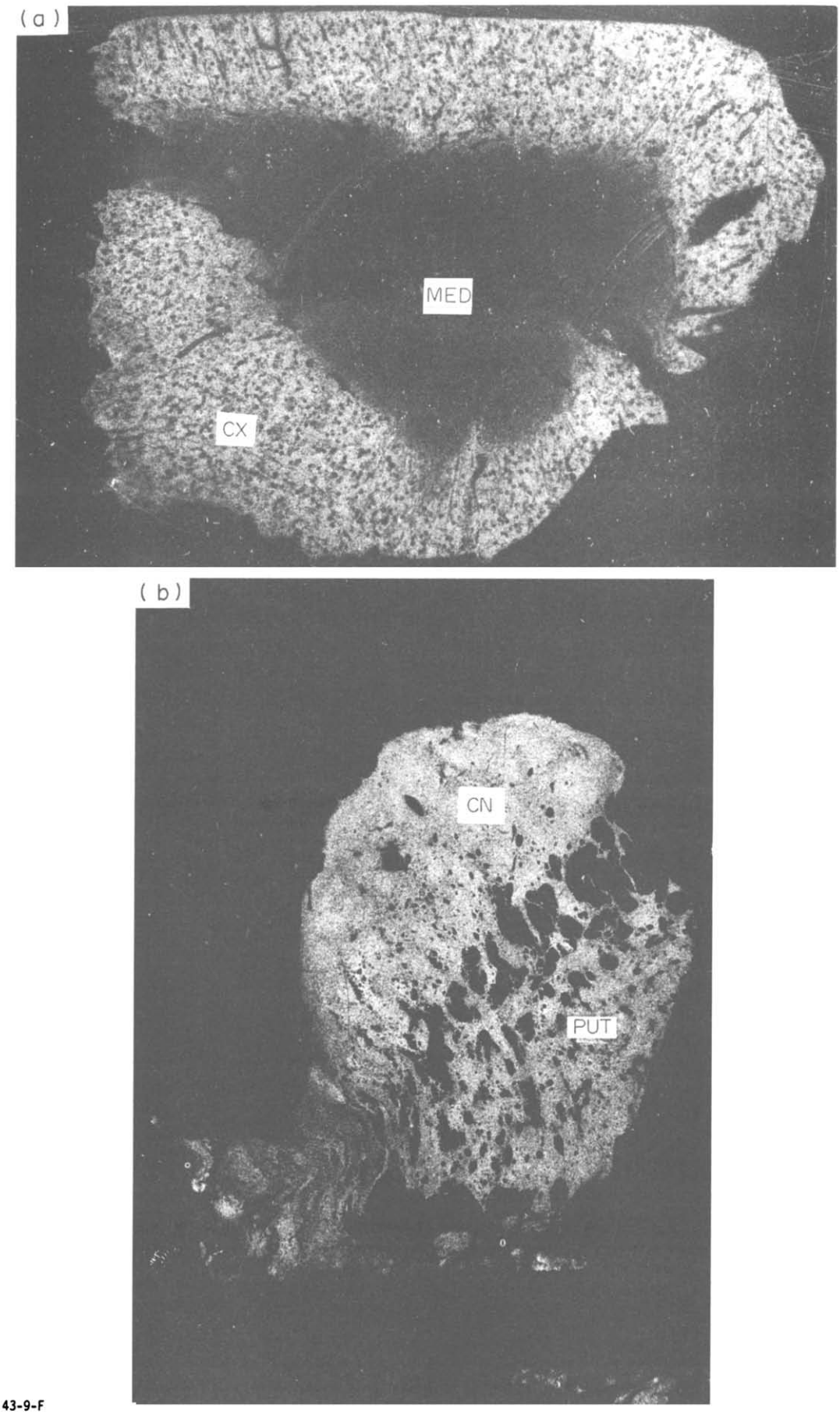
(a) Saturation analysis of [^{3}H]SCH23390 binding to renal tubular and glomerular membranes was carried out as described in Materials and Methods. (b) Inhibition constants were derived from competition curves versus [^{3}H]SCH23390 for each compound. Values are means of two experiments ± range or three experiments ± S.D.

the renal cortex suggests that binding could be to a protein which is involved in the excretion or metabolism of xenobiotics in the kidney. The uric acid transporter has a role in drug excretion in the kidney and this transporter can be inhibited by millimolar concentrations of lactic acid [32]. However, lactic acid at a concentration of 10^{-2} M had no activity at the [^{3}H]SCH23390 binding site (data not shown). The possibility of [^{3}H]SCH23390 binding to other transporters of xenobiotics or to an enzyme, such as cytochrome P450, responsible for the metabolism of drugs is another possibility. Recently it has been shown that [^{3}H]GBR-12935 binding in brain is partly to the dopamine transporter and partly to cytochrome P450 II D1 [33]. Similarly the dextromethorphan/sigma binding site may be the same cytochrome P450 II D1 [34]. Preliminary competition experiments with ligands selective for this enzyme showed that [^{3}H]SCH23390 binding in the renal cortex was not to cytochrome P450 II D1.

The data reported here are similar to those obtained using [^{3}H]SCH23390 [15] and [^{125}I]-SCH23982 [13] binding in rat renal cortex where the affinities of these radioligands were respectively 10- and 100-fold less than their affinities for binding to striatal D_1 dopamine receptors. The stereoselectivity for butaclamol binding was comparatively low in these studies (about 5-fold) [14] and the affinities of the sites for dopamine were also rather low (IC_{50} = 6.3 mM [14]). Thus, it seems that in these studies the same non-dopaminergic sites as described in the present study are being observed. By contrast, however, in human renal cortex [^{125}I]SCH23982 binding sites with very similar characteristics to brain D_1 dopamine receptors have been described [12]. The number of these sites (19 fmol/mg) was also much lower than the number of sites described in the present study (665 fmol/mg). There was no evidence in the present study for a minor population of sites corresponding to a conventional D_1 dopamine receptor. Thus, there may be significant species differences in the binding of radioactive benzazepines to kidney tissues.

The localization of [^{3}H]SCH23390 binding predominantly in the renal cortex rather than the medulla, as shown by the autoradiographic study on bovine kidney, is the same pattern of distribution observed in the rat kidney [14]. It is interesting that the dopamine- and cAMP-regulated phosphoprotein (DARPP-32) which localizes in the central nervous system to dopaminoceptive cells containing the D_1 dopamine receptor has been found, by *in situ* hybridization and immunocytochemistry, in the thick ascending limb of the loop of Henle which gives rise to a strong signal in the outer medulla and medullary rays with no signal in the renal cortex [35]. There also seems to be a species difference in the cellular localization of [^{3}H]SCH23390 and [^{125}I]SCH23982 binding within the renal cortex. In the rat kidney, autoradiographic studies revealed binding to tubules only [13, 14] whereas ligand autoradiography of human kidney [16] and the radioligand binding on tubular and glomerular membranes from bovine kidney in the present study indicate an equal distribution of [^{3}H]SCH23390 and [^{125}I]SCH23982 binding sites between tubules and glomeruli.

Fig. 3. Autoradiography of [^{3}H]SCH23390-labelled sections of (a) bovine kidney and (b) bovine basal ganglia. Cryostat sections (20 μM) were incubated with [^{3}H]SCH23390 (1 nM kidney; 0.5 nM basal ganglia) as described in Materials and Methods. The photomicrographs of the resultant autoradiograms show a high density of binding sites in renal cortex (CX), while no labelling above background was detected in renal medulla (MED, a). In the basal ganglia (b), as expected, a high density of binding to D_1 dopamine receptors in the caudate nucleus (CN) and putamen (PUT) was observed. Non-specific binding, determined by the addition of 1 μM unlabelled SCH23390 for renal cortex sections and 10 μM (+)-butaclamol for basal ganglia exposed autoradiograms no more intensely than film background.


(a)
MED
CX
(b)
CN
PUT

Resolution of the reasons for these species differences may aid in the determination of the function of these binding sites within the kidney.

In summary, by direct comparison of [^{3}H]-SCH23390 binding to bovine caudate nucleus and bovine renal cortical membranes we have shown the presence of a non-dopaminergic [^{3}H]SCH23390 binding site in renal cortex which has pharmacological characteristics distinct from those of a D_1 dopamine receptor. There are two possibilities for the identity of this binding site. The first is that [^{3}H]SCH23390 binding is to a protein involved in xenobiotic metabolism or excretion in the kidney which is suggested by the wide range of exogeneous compounds which are active at the binding site. The second possibility is that [^{3}H]SCH23390 binding is to the D_1 dopamine receptor protein modified such that the pharmacological characteristics are vastly different to those of the model D_1 dopamine receptor of striatum. The physiological reasons for such a modified receptor are unclear. Further studies, such as photoaffinity labelling or purification of the renal cortex binding site, would be required in order to determine the nature of the protein to which [^{3}H]-SCH23390 binds. However, this study does show that [^{3}H]SCH23390 binds not only to D_1 dopamine receptors and certain 5HT-receptor subtypes but also to an as yet unidentified non-dopaminergic binding site.

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