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SPECIFIC BINDING OF ^{125}I SCH 23982, A SELECTIVE DOPAMINE (D_1) RECEPTOR LIGAND TO PLASMA MEMBRANES DERIVED FROM HUMAN KIDNEY CORTEX

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Abstract—Binding of the selective D-1 dopamine receptor ligand ^{125}I SCH 23982 was studied using crude plasma membranes derived from human renal cortex. ^{125}I SCH 23982 bound saturably to a single high affinity site (K_d = 650 pM, B_{max} = 19 fmol/mg protein). Binding at 37° was rapid and reversible with forward and reverse rate constants of 5.79×10^8 min^{-1} m^{-1} and 0.156 min^{-1} respectively. Antagonist and agonist competition for ^{125}I SCH 23982 binding was also consistent with the existence of a single site possessing pharmacological characteristics similar to a D-1 dopamine receptor. It is suggested that this site may represent a D-1 (or DA_1) dopamine receptor present in human renal cortex.

Dopamine receptors in the central nervous system have been classified into D-1 and D-2 subtypes on the basis of their relationship to adenylate cyclase; stimulation of D-1 receptors activates adenylate cyclase [1], whereas stimulation of D-2 receptors has been reported to inhibit adenylate cyclase [2]. The functional role of central D-1 receptors is unclear at present [3]. In contrast, in the periphery, dopamine receptors linked to stimulation of adenylate cyclase have been shown to mediate vasodilation in a variety of blood vessels [4, 5] and are probably responsible for mediating the natriuretic effects of dopamine on the renal tubule [6]. Although these receptors defined in pharmacological studies have been generally classified as DA-1 rather than D-1 [7], they appear to bear considerable similarities to the central D-1 receptor, although some differences have been reported [8]. There have, however, been few studies investigating such DA-1 or D-1 dopamine receptors peripherally using ligand binding techniques. Brodde and colleagues [9, 10] reported results using ^{3}H spiroperidol in rabbit mesenteric and renal arterial membranes which they interpreted as representing binding to the D-1 like site, and Felder and coworkers [11] reported the existence of multiple ^{3}H haloperidol ligand binding sites in rat kidney, one of which they considered to be a D-1 site. Subsequently, a similar site was defined in rabbit proximal tubule [12]; however, given the poor selectivity of these agents for D-1 receptors it is unclear whether the sites defined in the above studies truly represent D-1 receptors.

Recently benzazepines such as SCH 23390 and congeners have been shown to be highly selective antagonists of the D-1 receptor [13] and have been used in ligand binding studies of this receptor [14]. ^{125}I SCH 23982 is an iodinated analogue of SCH 23390, which has been reported to be a selective ligand for D-1 receptors in rat striatum [15]. We have therefore used this agent to investigate whether D-1 sites are present in human renal cortex, a peripheral tissue which possesses functional dopamine D-1 receptors [16]. Some of these results have been presented previously to the British Pharmacological Society [17].

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MATERIALS AND METHODS

A crude plasma membrane fraction of human renal cortex was prepared by a modification of the technique described by Snavely and Insel [18]. Specimens of human kidney were obtained from five nephrectomies for renal carcinoma and one normal kidney obtained within 18 hr post-mortem. Macroscopically normal pieces of renal cortex were separated from medulla, and homogenised in 20× volume of buffer containing Tris–HCl (50 mM), sucrose (200 mM), EDTA (5 mM) and phenylmethyl sulphonyl fluoride (5 mM) (pH 7.4) at 4° by three 10 sec bursts of a Polytron (Kinematica GmbH). The homogenate was filtered through three layers of muslin and centrifuged at 500 *g* for 5 min to remove unhomogenised tissue. The supernatant was then centrifuged at 30,000 *g* for 30 min.

The resultant pellet was resuspended in buffer containing Tris–HCl (50 mM) and EDTA (5 mM) (pH 7.4) to an approximate protein concentration of 5 mg/ml. Aliquots of the crude membrane preparation were then either frozen in liquid nitrogen and stored at −70° for subsequent use or used immediately for studies. Prior to use, membrane aliquots were diluted in 30× volume of Billard's Tris buffer containing Tris–HCl (50 mM), NaCl (120 mM), KCl (5 mM), $CaCl_2$ (2 mM) and $MgCl_2$ (1 mM) (pH 7.4), centrifuged at 30,000 *g* and finally resuspended in Billard's Tris buffer to give an approximate protein concentration of 2 mg/ml for use in binding studies. All binding studies were performed in polypropylene tubes in duplicate. Standard binding assays were performed in a volume of 300 μl assay buffer comprising 100 μl tissue homo-

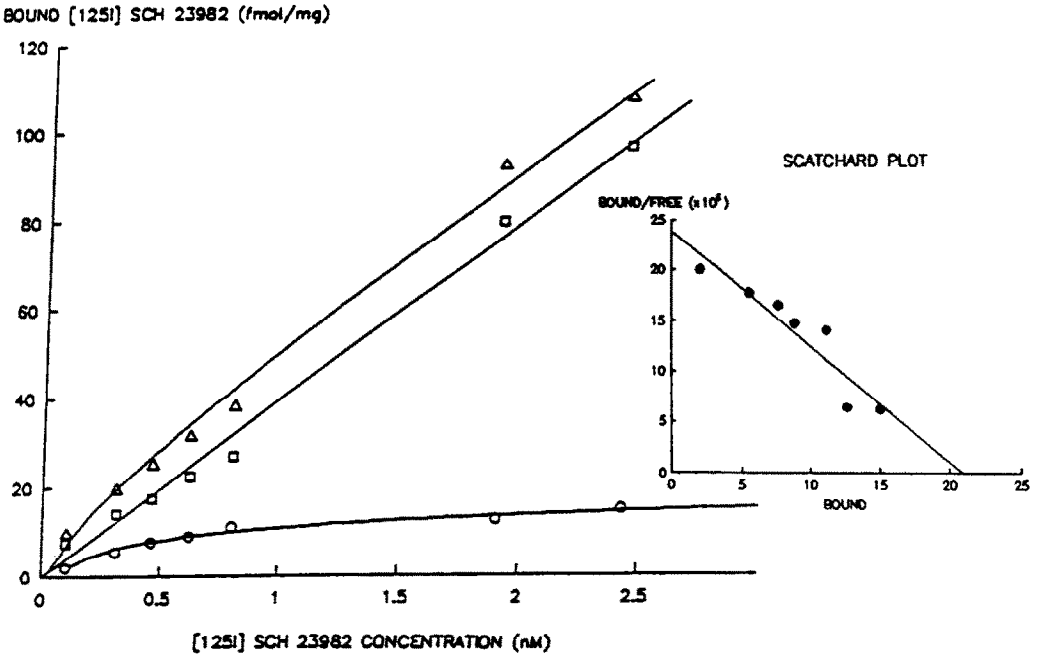

Fig. 1. Representative saturation isotherm showing binding of ^{125}I SCH 23982 to renal cortical plasma membranes: △, total binding; □, non-specific binding; ○, specific binding.

genate, 100 μl drugs and 100 μl radiolabelled ^{125}I SCH 23982 (~300 pM final concentration). Pargyline (10 μM) was included in assays when dopamine was the displacing drug to inhibit monoamine oxidase. For studies at equilibrium, the assay was incubated at 37° for 45 min. The assay was terminated by filtration through Whatman GF/B filter paper using a Brandel 24-well cell harvester. Each filter paper was washed three times with 1.5 ml ice cold 50 mM Tris buffer. Filter paper was allowed to dry in air before Gamma counting. Filter paper had been soaked in 0.3% polyethyleneimine at 4° overnight as preliminary studies showed this procedure reduced filter paper binding of ligand.

Non-specific binding was defined as binding in the presence of 1 μM SCH 23390. For saturation studies increasing concentrations of ligand (1 pM–3 nM) were incubated with membranes to equilibrium. In displacement studies, the standard ligand concentration was approximately 300 pM. For association studies, membranes were incubated with ^{125}I SCH 23982 (~300 pM) at 37° and incubation terminated by filtration at various time points following addition of membranes. The dissociation rate constant was derived by addition of unlabelled SCH 23982 (1 μM) to assays at equilibrium and measuring the dissociation of ^{125}I SCH 23982 from the receptor with respect to time.

The protein content of the plasma membranes was assayed using a commercially available Coomassie Blue G-250 based reagent (Pierce Protein Assay Reagent) with bovine serum albumin as standards.

Data from binding studies were analysed using a weighed non-linear least squares fitting program (LIGAND) modified for use on a IBM XT microcomputer [19]. Data from association and dissociation studies were also analysed using a non-linear fitting program (KINETIC) [19].

Drugs

Drugs used in the study were (+) and (−) butaclamol (Research Biochemicals Inc., U.S.A.), dopamine (Sigma Chemical Co., Poole, U.K.), domperidone (Janssen Life Sciences Products, U.K.), fenoldopam (a gift from Smith Kline and French Ltd., Philadelphia, PA), ketanserin (a gift from Janssen Ltd., U.K.), pargyline (Sigma), phentolamine (Sigma), polyethyleneimine (Sigma), ^{125}I SCH 23982 (New England Nuclear, Boston, MA), SCH 23390 (a gift from Schering Plough Corp., U.S.A.), SCH 23388 (Research Biochemicals Inc., U.S.A.), (R) and (S) Sulpiride (gifts from Ravizza SpA).

RESULTS

^{125}I SCH 23982 bound to a crude plasma membrane preparation of human renal cortex. Total bound ^{125}I SCH 23982 under the assay conditions represented less than 10% total free ^{125}I SCH 23982. Specific binding (defined as that displaceable by 10^{-6} M SCH 23390) represented 50% of total ^{125}I SCH 23982 bound. Specific binding of ^{125}I SCH 23982 was linearly related to protein concentration over the range 0.24–3.8 mg/ml.

Specific binding of ^{125}I SCH 23982 was saturable and consistent with binding to one site. A representative saturation curve taken from one experiment is shown in Fig. 1. Analysis of three such curves from separate experiments using renal cortex from different individuals gave an affinity constant (K_d) = 650 ± 270 pM and a maximum binding capacity (B_{max}) of 19.0 ± 8.7 fmol/mg protein

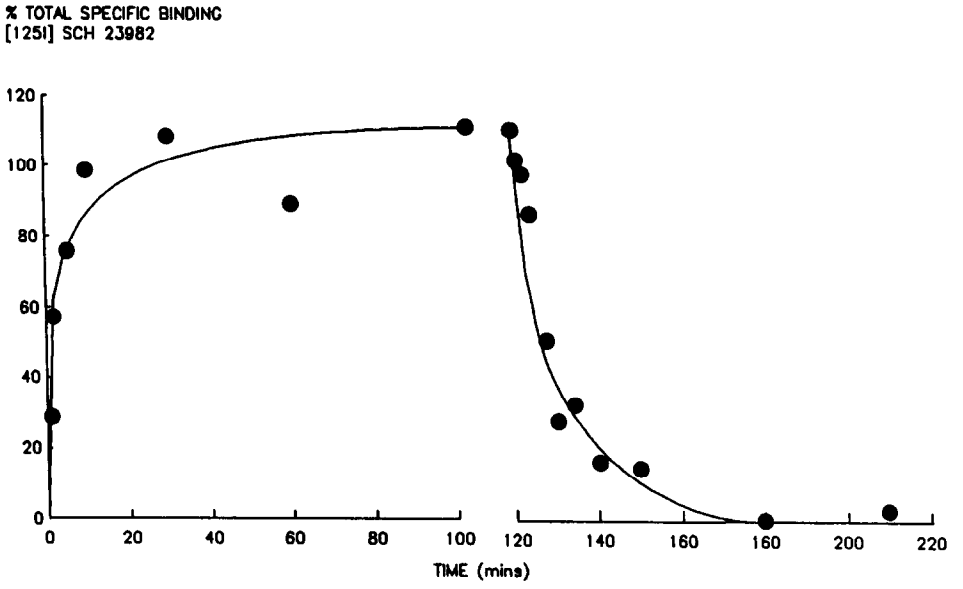

Fig. 2. Association and dissociation characteristics of ^{125}I SCH 23982 binding to human renal cortex. Points represent means derived from three separate experiments.

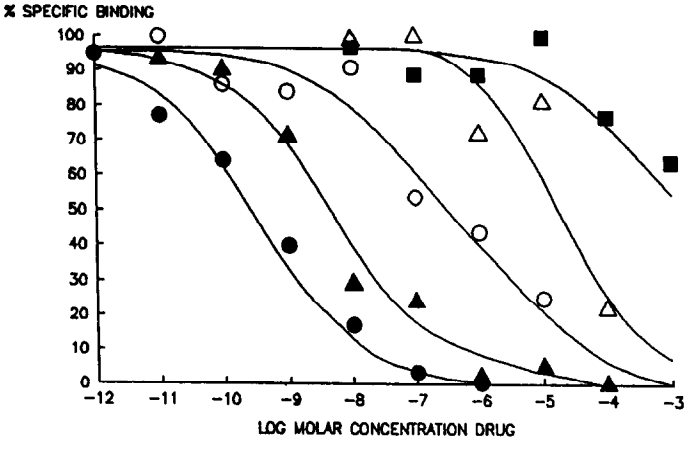

Fig. 3. The effect of various antagonist drugs on ^{125}I SCH 23982 binding to human renal cortex. Points represent mean values derived from 2–3 individual experiments: ●, SCH 23390; ○, SCH 23388; ■, (R) sulpiride; △, ketanserin; ▲, (+) butaclamol.

(mean ± SEM) by non-linear regression. Equivalent values by Scatchard analysis were $K_d = 1.11 \pm 0.59$ nM and $B_{max} = 25.7 \pm 9.6$ fmol/mg protein.

These results compare with a value for the K_d derived from kinetic studies of 280 pM (Fig. 2). Association of ^{125}I SCH 23982 reached equilibrium within 20 min with an estimated K_{+1} of $5.79 \pm 1.9 \times 10^8$ min^{-1} M^{-1} and was interpreted as representing binding to a single site. Similarly analysis of dissociation data was not significantly improved by postulating the existence of more than one site and K_{-1} was calculated to be 0.156 ± 0.14 min^{-1}. Subsequent studies at equilibrium were routinely conducted using an incubation time of 45 min.

The results of various studies using increasing concentrations of unlabelled drugs to displace labelled ^{125}I SCH 23982 from its binding sites on human renal cortex are shown in graphical form in Figs 3 and 4 and the derived K_i values from these studies are shown in Table 1.

Analysis of the displacement curve for drugs gave Hill slopes not significantly different from unity for all those agents displacing ^{125}I SCH 23982 with the exception of SCH 23388, dopamine and fenoldopam, for which Hill slopes were significantly less than unity.

DISCUSSION

These studies indicate the existence of binding

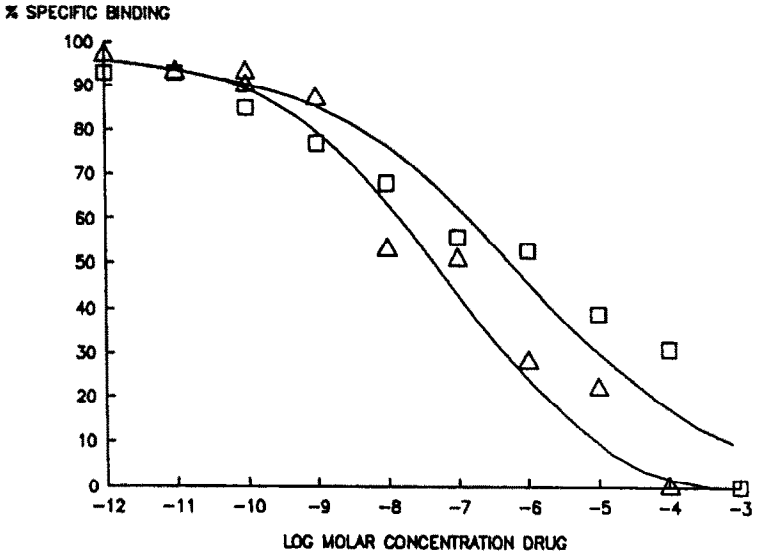

Fig. 4. The effect of dopamine receptor agonists on ^{125}I SCH 23982 binding to human renal cortex. Points represent mean values derived from three individual experiments: □, dopamine; △, fenoldopam.

Table 1. Displacement of ^{125}I SCH 23982 binding to human renal cortical plasma membranes by various drugs

Drug	K_i (mean ± SEM) (M) of drug at ^{125}I SCH 23982 binding site in human renal cortex	n
Antagonists		
(+) SCH 23390	$3.4 \pm 3.1 \times 10^{-10}$	3
(−) SCH 23388	1.7×10^{-5}*	2
(+) Butaclamol	$2.0 \pm 2.0 \times 10^{-8}$	3
(−) Butaclamol	$>10^{-4}$	2
Ketanserin	1.8×10^{-5} M	2
Phentolamine	$>10^{-4}$	2
Domperidone	$>10^{-4}$	2
(*R*) Sulpiride	1.2×10^{-3}	2
(*S*) Sulpiride	$>10^{-3}$	2
Agonists		
Dopamine	$1.5 \pm 1.5 \times 10^{-5}$*	3
Fenoldopam	$7.6 \pm 3.5 \times 10^{-8}$*	3

* Hill slope significant < 1.

sites for ^{125}I SCH 23982 in human kidney with pharmacological characteristics consistent with its being a D-1 dopamine receptor. This binding site is similar to D-1 sites previously described using ^{125}I SCH 23982 or ^{3}H SCH 23390 in rat striatum [15] and human caudate nucleus [20].

The unitary pseudo Hill slopes for ^{125}I SCH 23982 binding and the kinetics of binding suggest that binding is to a single site. Similarly, the unitary pseudo Hill slopes of displacing antagonists are consistent with a one site model. The low pseudo Hill slopes found when dopamine and fenoldopam were used as displacing agents is unsurprising. Similar results have been reported for agonist displacement curves using ^{3}H SCH 23390 as a D-1 ligand in rat brain [21–23] and is a recognised feature of agonist interaction with receptors which may be coupled to guanine nucleotide regulatory subunits [24]. Further studies investigating the effects of GTP or GTP analogues would therefore be interesting in this tissue. The explanation of the low pseudo Hill slope obtained when SCH 23388, the enantiomer of SCH 23390, was the displacing agent is unclear; whether this agent possesses weak partial agonist activity or displaces SCH 23982 from non-specific sites at high concentrations is not known.

In view of the finding of D-1 like sites in human renal cortex, it is tempting to suggest that these sites may represent D-1 or DA-1 receptors in this location. Dopamine receptors linked to generation of cAMP have been demonstrated in renal tissue derived from several species including man [11, 25, 26]; activation of these receptors increases renal blood flow by an action on the renal vasculature [6], produces natri-

uresis by a direct tubular action [16], and may be involved in salt and water handling by the kidney [6, 27]. Further studies of D-1 receptor induced cAMP accumulation are currently being undertaken to establish whether the ligand binding site defined in this investigation does represent the renal D-1 receptor.

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