Synthesis and Applications of an Aldehyde-Containing Analogue of SCH-23390

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SCH-23390 is a high-affinity antagonist selective for D1 dopamine receptors (K_i = 2.5 nM). It does not contain a functional group that can be conveniently coupled to commercially available resins for affinity chromatography or to prepare photolabels for photoaffinity labeling of receptors. To construct an affinity resin for purification of dopamine D1 receptors, an aldehyde analogue of SCH-23390, (±)-7-chloro-8-hydroxy-1-(4′-formylphenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (ASCH), was synthesized. 8-Methoxy-1-(4′-bromophenyl)-SCH-23390 was lithiated, formylated, and O-demethylated to form the aldehyde. NMR and IR analyses were performed to characterize the product. Assays were performed with the radioligand [^{125}I]SCH-23982 to define the biological activity of the aldehyde. ASCH displaced [^{125}I]SCH-23982 binding from caudate membranes with a K_i value of 7.1 nM. ASCH has been coupled through the aldehyde group on the phenyl ring to diaminodipropylamine–agarose for affinity chromatography. After solubilization of caudate membranes in 1% digitonin, the affinity resin retained binding sites for [^{125}I]SCH-23982 that were eluted with 10 mM SCH-23390. The aldehyde was also covalently coupled to biotin hydrazide for fluorescence labeling of dopamine D1 receptors. The biotin-conjugated aldehyde of SCH-23390 displaced [^{125}I]SCH-23982 binding from caudate membranes with a K_i value of 9.3 nM.

Dopamine receptors have been traditionally divided into two pharmacologically distinct classes, D1 and D2. Dopamine D1 receptors have been linked to stimulation of adenylyl cyclase activity (Kebabian & Calne, 1979; Kebabian et al., 1984) and stimulation of phosphoinositide hydrolysis (Felder et al., 1989) or not linked to adenylyl cyclase activity in some systems (DeKeyser et al., 1989). Additionally, D1 receptors were classified by displaying high affinity for the benzazepine derivatives SCH-23390, a receptor antagonist (Iorio et al., 1983), and SKF-38393, a receptor agonist (O'Boyle & Waddington, 1984). D2 receptors have been linked to inhibition of adenylyl cyclase activity (Kebabian et al., 1984) and display high affinity for a variety of compounds including neuroleptics of varying structures (Seeman et al., 1976). Recently the genetic and cDNA sequences of adenylyl cyclase linked human (Sunahara et al., 1990; Dearry et al., 1990; Zhou et al., 1990) and rat (Monsma et al., 1990) D1 receptors were reported. The elucidation of these sequences increases the methods available for studying D1 receptor structure and regulation. However, the D1 receptor has never been purified, and the appropriate start site of the D1 coding sequence is still a subject of debate.

Thus far, purification of dopamine D1 receptors has been incomplete. Solubilization of striatal membranes with 1% digitonin in a buffer with high ionic strength followed by lectin affinity chromatography led to the isolation of a binding site for SCH-23390 that copurified with a guanine nucleotide-binding protein (Niznik et al., 1986). Solubilization of D1 receptors with sodium cholate and partial purification using a sulfhydryl affinity column have been described, but reconstitution of binding sites into phospholipid vesicles was necessary for detection of binding activity (Sidhu, 1990). Gingrich et al. (1988) reported the synthesis of an affinity resin for D1 receptors that involved coupling a 4′-aminophenyl derivative of SCH-23390 to an activated agarose resin. The affinity resin retained binding sites for SCH-23390 that were specifically eluted with (+)-butaclamol, but amino acid sequence data were not reported.

Autoradiographic studies of the binding of radiolabeled D1 antagonists [^{3}H]SCH-23390 and [^{125}I]SCH-23982 to brain sections have revealed a high density of D1 receptors in the caudate nucleus, nucleus accumbens, and substantia nigra (Boyson et al., 1986; Dawson et al., 1988). Autoradiographic techniques do not, however, allow localization of dopamine receptor subtypes at the cellular or subcellular level (Schwarcz et al., 1978; Trugman et al., 1986; Palacios, 1986). Ariano et al. (1989) have recently reported the development of D1- and D2-selective receptor antagonists coupled directly to fluorescein, which may permit histofluorescent localization of these receptors. Final resolution of controversies surrounding dopamine receptor localization awaits the development of probes that can be used at the level of the electron microscope.

To develop a new, specific probe for the study of D1 receptors, we have synthesized a 4′-formylphenyl analogue of SCH-23390 (ASCH) that is easily coupled to primary amines through an aldehyde moiety. By linking the aldehyde to an amine-activated agarose resin, ASCH has been used to create an affinity resin for D1 receptors. We have also covalently coupled ASCH to biotin hydrazide, creating a D1 receptor ligand for use with avidin-linked compounds including fluorescein, rhodamine, horseradish peroxidase, and ferritin. The coupling of ASCH to biotin hydrazide increases the number of techniques that may potentially be used to study the distribution of D1 receptors. Derivatives of ASCH may ultimately permit resolution of the distribution of D1 receptors at the cellular level.

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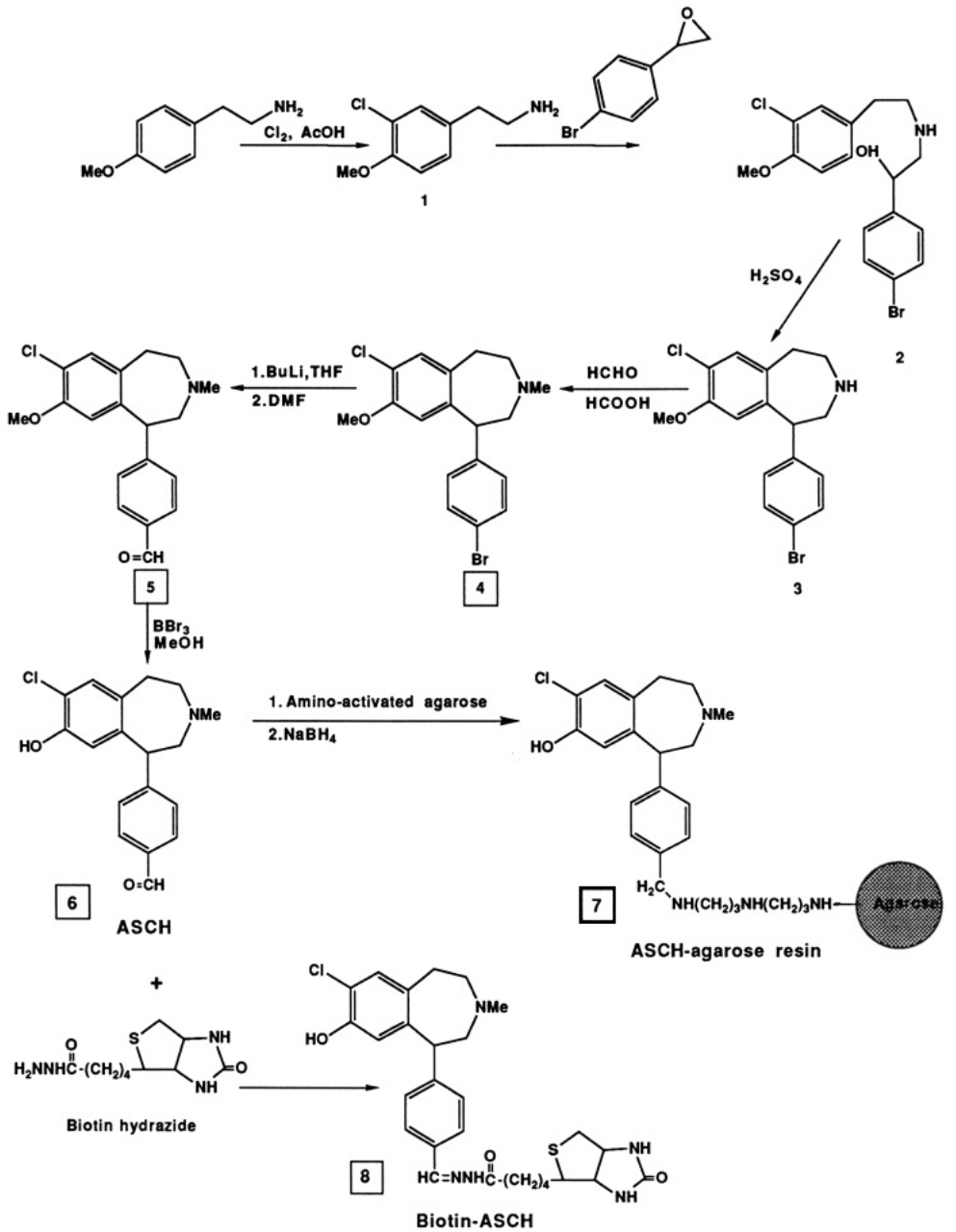
Scheme I. Synthetic Pathway for a 4′-Formylphenyl Analogue of SCH-23390, ASCH (6)[a]



[a] ASCH was subsequently coupled to an amino-terminal agarose resin to produce a D1-receptor affinity resin (7) and to biotin hydrazide to produce a biotinylated analogue, biotin–ASCH (8).

EXPERIMENTAL PROCEDURES

Chemical Syntheses (Scheme I). The synthesis of (±)-7-chloro-8-methoxy-1-(4′-bromophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (compound 4) was based on a protocol described by Wyrick and Mailman (1985) as utilized by Chumpradit et al. (1989).

Proton nuclear magnetic resonance spectroscopy (NMR) was recorded on a Varian EM 360A spectrometer. The chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Infrared spectra were obtained with a Mattson Polaris FT-IR spectrometer. Melting points were determined with a Meltemp apparatus and are reported uncorrected for compounds 5 and 6. Fast-atom bombardment mass spectrometry was performed in the Department of Chemistry at the University of Pennsylvania, and all values are within 18 ppm of the theoretical values.

(±)-7-Chloro-8-methoxy-1-(4′-formylphenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (Compound 5). Compound 4 (5 g, 13 mmol) was added to 100 mL of dried tetrahydrofuran (THF) and cooled to −78 °C in a dry ice–acetone bath. *n*-Butyllithium (8.1 mL, 13 mmol) was added with stirring over 2 min, yielding a solution with a deep red color; 2.0 mL of dimethylformamide was added and the solution was stirred at −78 °C for 1 h. The reaction was quenched by the addition of 5 mL of saturated ammonium chloride solution and allowed to warm to room temperature. THF was removed under reduced pressure and the residue was extracted with dichloromethane, dried over anhydrous sodium sulfate, and separated on a silica gel column eluted with a mixed solvent (dichloromethane–methanol–ammonium hydroxide 95:5:0.1) to yield 1.98 g of compound 5 (48% yield). The resulting product was further purified by recrystallization. Sodium bisulfite was added to 75% ethanol until the solution became cloudy and then water was added slowly until the mixture became clear. This solution was added directly to 1.98 g of reaction product and the

resulting crystals were filtered and dissolved in freshly prepared, saturated potassium carbonate. The mixture was extracted with dichloromethane and the solvent evaporated under reduced pressure to yield 0.75 g of compound **5** (18% overall yield): mp 134–140 °C; FT-IR (KBr) ν 1690 (s, C=O) 1600, 1570, 1500, 850, 830, 800 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.95 (s, 1 H, HC=O), 7.90, 7.75, and 7.37, 7.22 (AA'BB', 4 H, ArH'), 7.10 (s, 1 H, ArH-6), 6.30 (s, 1 H, ArH-9), 4.50–4.25 (m, 1 H, CH), 3.65 (s, 3 H, OCH_3), 3.20–2.55 (m, 6 H, $(CH_2)_3$), 2.40 (s, 3 H, NCH_3) ppm; MS (FAB) $(M + H)^+$ 330.1248, calcd for $C_{19}H_{20}O_2NCl$ 330.1261.

(±)-7-Chloro-8-hydroxy-1-(4'-formylphenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (6, ASCH). (Formylphenyl)benzazepine **5** (100 mg, 0.303 mmol) in 10 mL of dried dichloromethane was cooled in a dry ice-2-propanol bath under argon. Boron tribromide (0.9 mL, 0.9 mmol) was added dropwise with stirring and the reaction was allowed to warm to room temperature with stirring for 2 h. Volatile substances were evaporated under nitrogen, after which 10 mL of methanol was added to the reaction mixture and stirred overnight at room temperature. Methanol was then removed under reduced pressure and the residue stirred with water. The pH of the mixture was increased with 10% NaOH and the precipitate filtered. The filtrate was adjusted to pH 7 with dilute HCl and extracted three times with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The product ASCH (**6**; 30 mg, 32% yield) was recrystallized overnight in 100% ethanol. A C18 column for reverse-phase HPLC and isocratic elution with a mixture of 50% acetonitrile, 10 mM ammonium formate, pH 6.5, at a flow rate of 1 mL/min and a retention time of 4.45 min purified compound **6** (>99% purity): mp 170–172 °C; UV λ_{max} 256 nm (ϵ 5.9 × 10^3 $M^{-1}\cdot cm^{-1}$); FT-IR (KBr) ν 2300–3300 (br OH) 1690 (C=O), 1600, 1570, 1500, 850, 830, 800 (para substituted phenyl) cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.95 (s, 1 H, HC=O), 7.95, 7.8, and 7.47, 7.33 (AA'BB', 4 H, ArH'), 7.10 (s, 1 H, ArH-6), 6.30 (s, 1 H, ArH-9), 4.55–4.30 (m, 1 H, CH), 3.27–2.60 (m, 6 H, $(CH_2)_3$), 2.40 (s, 3 H, NCH_3); MS (FAB) $(M + H)^+$ 316.1036, calcd for $C_{18}H_{18}O_2NCl$ 316.1104.

ASCH–Agarose Affinity Resin. Diaminodipropylamine–agarose (1 mL; Pierce, Rockford, IL) containing 10 μmol of primary amine was equilibrated into 100% ethanol by gradually increasing the concentration of ethanol over 10 washes of 10 mL each. ASCH (6.3 mg, 20 μmol) dissolved in 1 mL of 100% ethanol was incubated at room temperature overnight with the resin while the mixture was rotated end over end to insure constant mixing (setting 3, multipurpose rotator Model 151, Scientific Industries). Because the ligand was coupled to the resin through an imine bond, the ligand-coupled resin was washed with ethanol (200 mL) and reduced overnight with sodium borohydride (1.9 mL, 50 μmol) in ethanol (1 mL) at room temperature with rotation. The resin was washed extensively with 100% ethanol and gradually brought to 100% water by decreasing the concentration of ethanol in a series of washes. The resulting gel was washed thoroughly with water and stored.

Biotin–ASCH. Biotin hydrazide (50 mg, 0.092 mmol) and ASCH (29 mg, 0.092 mmol) were dissolved in 10 mL of methanol and refluxed overnight with stirring. The solvent was evaporated under reduced pressure and the resulting product purified by silica gel chromatography (dichloromethane–methanol–ammonium hydroxide 90:10:0.1).

Preparation of Tissue. Rabbit brains obtained on dry ice from Pel-Freeze (Rogers, AK) were thawed on ice and the striata were removed and placed in 50 mM Tris, pH 7.4, containing 10 mM EDTA, 150 mM NaCl, and a mixture of protease inhibitors [1 μg/mL of soybean trypsin inhibitor, leupeptin, aprotinin, and 10 μg/mL of phenylmethanesulfonyl fluoride (PMSF)] at 10 mL/mg wet weight of striata. The tissue was homogenized with a Polytron homogenizer (setting 6) for 10 s, and membranes were pelleted by centrifugation for 10 min at 10000*g*. This was repeated twice and the tissue homogenate at 100 mg/mL was frozen in aliquots of 10 mL at −70 °C until needed.

Binding Assays for D1 Receptors. For assays with striatal membranes, tissue homogenates were thawed on ice and centrifuged at 10000*g* for 10 min. Pellets were suspended in 50 mM Tris, pH 7.4, containing 10 mM EDTA (ethylenediaminetetraacetic acid), 150 mM NaCl, 0.1% BSA (bovine serum albumin), and protease inhibitors as described above (buffer I) at 0.5 mg of tissue/mL of buffer. [^{125}I]SCH-23982 (New England Nuclear, Du Pont, Boston, MA) was used to assay for D1 receptors in a total assay volume of 0.25 mL with 0.1 mL of striatal membranes and 0.2 nM [^{125}I]SCH-23982 in buffer I. SCH-23390 (50 μM) was used to define nonspecific binding. Binding assays were carried out for 18 h at 4 °C and incubations were terminated by the addition of 5 mL of ice-cold 10 mM Tris containing 0.9% NaCl, pH 7.4 (wash buffer), followed by filtration over glass-fiber filters (#30, Schleicher and Schuell, Keene, NH) with two washes of 5 mL of wash buffer.

For binding assays on solubilized proteins, 0.2 mL of column eluate was used in a 1-mL assay with 0.2 nM [^{125}I]-SCH-23982. SCH-23390 (50 μM) was used to define nonspecific binding. Digitonin (0.1% w/v) was included in buffer I for assays of solubilized receptor. G-50 columns were used to separate bound radioligand from free. A 4-mL column of G-50 was equilibrated with buffer I plus 0.1% digitonin. A 1-mL sample was applied to each column and washed with 2.5 mL of buffer. The initial eluate (1.5 mL) was discarded and the next 2 mL collected.

Data Analysis. Competition curve-fitting was done by nonlinear least-squares regression analysis using the mathematical modeling program NEWFITSITES on the NIH-sponsored PROPHET system (Lin et al., 1987).

Solubilization of Membrane Proteins. Tissue homogenates were thawed on ice and centrifuged at 10000*g* for 10 min. Pellets were suspended at 0.2 g/mL in 100 mM Tris (pH 7.4) and 20 mM EDTA containing protease inhibitors. An equal volume of 2% (w/v) digitonin was added to the tissue preparation and incubated on ice with gentle agitation for 1 h. Insoluble material was sedimented by centrifugation for 1 h at 100000*g* at 4 °C, and the supernatant was collected and used immediately.

Affinity-Resin Purification Protocol. Solubilized membranes (1 mL) were applied to columns containing either the affinity resin or uncoupled agarose. The columns were capped and rotated overnight at 4 °C to allow receptors to bind. Columns were washed with 9 mL of 0.1% digitonin in 50 mM Tris, pH 7.4, and 10 mM EDTA, and the eluate was collected in three fractions each containing 3 mL. Columns were then incubated with buffer including 500 mM NaCl at 4 °C for 12 h with rotation and washed with 9 mL of the high-salt solution, and again the eluate was collected. The resins were then incubated with 10 mM SCH-23390 in the presence of 500 mM NaCl overnight at 4 °C and washed with 9 mL of the SCH-23390 solution. Each fraction was applied to a G-50 column for desalting as described for binding to solubi-

lized proteins above. The eluate was assayed for the presence of D1 receptors.

HRP Assay for D1 Receptors. Homogenates were thawed on ice and centrifuged at 10000*g* for 10 min. The pellets were resuspended in phosphate buffer (138 mM NaCl, 4.1 mM KCl, 5.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 11.1 mM glucose, pH 7.4) containing 3% BSA. Tissue (100 μg) was incubated at 37 °C for 90 min with or without 50 μM biotin–ASCH. SCH-23390 (50 μM) was added to one set of tubes to define nonspecific binding. Assay tubes were centrifuged at 4 °C for 2 min at 14000*g* and the supernatant was discarded. Pelleted membranes were resuspended in 250 μL of phosphate buffer containing 1 μg/mL avidin-linked horseradish peroxidase (HRP) and 3% BSA and incubated on ice for 15 min. Tubes were centrifuged for 2 min, washed once with 250 μL of phosphate buffer containing 3% BSA, and resuspended in 250 μL of phosphate buffer. Soluble HRP stain (250 μL; TMB peroxidase EIA substrate, Bio-Rad, Rockville Center, NY) was added to each assay tube and incubated for 2 min on ice before quenching with 100 μL of 1 N H_2SO_4. The optical density of each sample was read at 450 nm.

Protein Determinations. Protein concentrations were determined by the method of Bradford (1976) using bovine γ globulin as a standard.

RESULTS

The four-step synthesis of 4′-bromo benzazepine **4** followed a protocol established by Wyrick and Mailman (1985) (Scheme I) as described by Chumpradit et al. (1989). The overall yield is low (9%) because of the low yield of the chlorination reaction and the production of a trialkylamine side product along with product **2**. 4′-Bromo benzazepine was purified over a silica gel column prior to lithiation with *n*-butyllithium to replace the 4′-bromo group, producing an intensely red benzylic anion intermediate. Addition of 2 equiv of dimethylformamide (DMF) led to formation of 4′-formyl benzazepine **5**, which was purified by silica gel chromatography to remove excess DMF and recrystallized as a sodium bisulfite adduct. The final product, **6** (ASCH), was prepared by O-demethylation of purified **5** with boron tribromide.

ASCH inhibited the binding of [^{125}I]SCH-23982 to rabbit caudate membranes with a K_i value of 7.1 nM. The affinity of the receptors for ASCH was almost as high as that for SCH-23390 (K_i = 2.5 nM), and the inhibition curve conformed to a one-site fit as analyzed by nonlinear-regression analysis using the PROPHET system (Figure 1). ASCH was coupled to diaminodipropylamine–agarose as described in the Experimental Procedures to obtain an affinity resin for D1 receptors (Scheme I, compound **7**). UV analysis of the ethanol column washes after coupling showed a recovery of 15 μmol of ASCH, suggesting that approximately 50% of the resin-bound amine was coupled to ASCH. Final washes with ethanol revealed no free ASCH detectable by UV absorption spectroscopy. Prior to use, the affinity resin was washed extensively in 100% ethanol.

Binding sites for [^{125}I]SCH-23982 were eluted from the uncoupled agarose control column in the no-salt and high-salt washes (Figure 2). No additional binding sites were eluted by the addition of 10 mM SCH-23390, and recovery of binding sites for [^{125}I]SCH-23982 from the control column was nearly 100%. However, ca. 30% of the binding sites applied to the affinity resin could not be accounted for. They may have remained attached to the affinity resin after all washes or been destroyed during the absorption and elution procedures. In contrast to the control column, only 20% of the binding sites added to the affinity resin were eluted in no-salt and 500 mM NaCl washes. An additional 50% of the sites were eluted with 10 mM SCH-23390. The protein profile (data not shown) of ASCH–agarose affinity-resin washes showed that 25% of the total protein was eluted with 10 mM SCH-23390, which indicated a 2-fold purification of D1 receptors after affinity-resin chromatography.

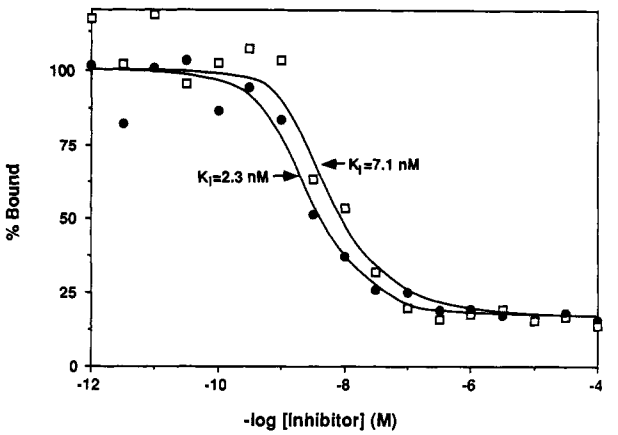


Figure 1. Inhibition of binding of [^{125}I]SCH-23982 to rabbit striatal membranes by ASCH (□) and SCH-23390 (●). The data shown are representative of results obtained in three similar experiments.

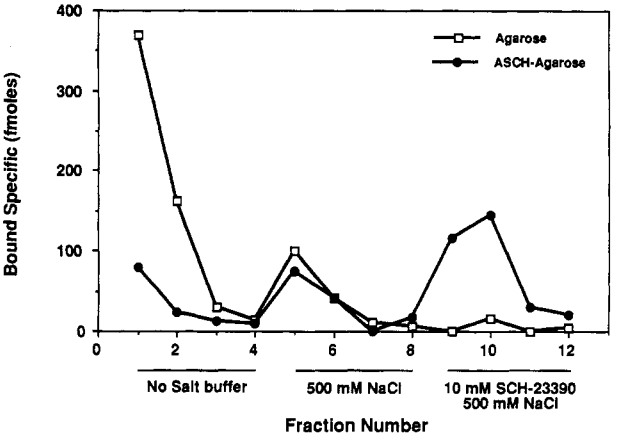


Figure 2. Elution profile of D1 receptors from ASCH–agarose resin (●) and uncoupled amine–agarose resin (□). Resins were incubated overnight with digitonin-solubilized rabbit striatal membranes and then washed and assayed for binding of [^{125}I]-SCH-23982 to solubilized receptors. The resins were first washed with 50 mM Tris containing 10 mM EDTA, 0.1% digitonin, and protease inhibitors (no salt buffer) at 4 °C. The second wash was with the same buffer to which 500 mM NaCl had been added. The third wash included 500 mM NaCl and 10 mM SCH-23390 (in 0.01% ethanol). The profile is representative of results obtained in two similar experiments.

ASCH was conjugated to biotin hydrazide in an attempt to create a probe for D1 receptors that could be coupled through avidin to many kinds of markers (fluorescent molecules, colloidal gold, horseradish peroxidase, etc.). Biotin–ASCH (Scheme 1, compound **8**) was synthesized as described in the Experimental Procedures and was shown to inhibit the binding of [^{125}I]SCH-23982 with a K_i value of 9.3 nM. The inhibition curves conformed to a one-site fit as analyzed by nonlinear-regression analysis on the PROPHET system. Biotin hydrazide at concentrations of up to 10 μM did not inhibit binding of the radioligand to D1 receptors.

Experiments with biotin–ASCH were performed to determine whether avidin would bind to the biotin portion

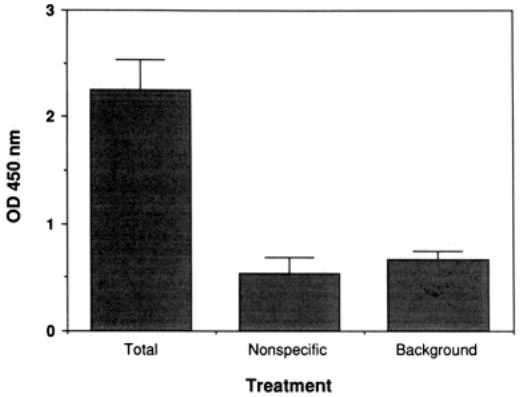


Figure 3. HRP assay for D1 receptors on rabbit striatal membranes using biotin–ASCH. Rabbit striatal membranes were incubated with 50 μM biotin–ASCH (total), biotin–ASCH plus 50 μM SCH-23390 (nonspecific), or assay buffer alone (background). Samples were then incubated with 1 μg/mL of avidin–HRP. The membranes were sedimented to separate bound ligand from free ligand and resuspended in HRP substrate. The resultant color reaction was quantitated by measuring optical density (OD) at 450 nm. The data points represent the average of triplicate determinations with standard deviations and are representative of results obtained in three similar experiments.

of the molecule prebound to D1 receptors. An assay based on soluble horseradish peroxidase substrate and avidin-linked HRP was devised. Avidin–HRP, as assayed by the optical density of the soluble HRP substrate, bound to striatal membranes in the presence of biotin–ASCH (Figure 3). HRP staining was specifically blocked by the presence of SCH-23390. Thus, biotin–ASCH was capable of binding specifically to dopamine D1 receptors while simultaneously binding to avidin.

DISCUSSION

An analogue of the selective D1 receptor antagonist SCH-23390 that contains an aldehyde group at the 4′-phenyl position has been synthesized. The aldehyde was positioned on a phenyl ring distant from the portion of the molecule shown to be important for high-affinity binding to D1 receptors (McQuade et al., 1988). ASCH inhibited binding of [^{125}I]SCH-23982 with a K_i value of 7.1 nM, which is only 3-fold higher than the K_i value for SCH-23390 (2.5 nM). The synthesis involved an extension of the pathway established for the synthesis of SCH-23390. Addition of the aldehyde group was accomplished by an efficient reaction scheme through a lithium anion exchange, as suggested by Chumpradit et al. (1989). The cyclization step proceeded with good yield and did not involve the use of hydrogen fluoride or another strong acid. An analogue of SCH-23390 containing a primary amine has previously been synthesized through reduction of a nitro group at the 4′-phenyl position (Gingrich et al., 1988). The presence of the nitro group at the 4′-phenyl position makes cyclization much more difficult (S. Chumpradit, unpublished observation). ASCH was readily coupled to primary amines in dimethyl sulfoxide (unpublished observation), ethanol, or methanol to yield an affinity resin and biotinylated derivatives of ASCH.

Preliminary applications of ASCH to studies of D1 receptors included construction of an affinity resin that selectively retained binding sites for [^{125}I]SCH-23982. The affinity resin specifically retained approximately 80% of the binding sites for [^{125}I]SCH-23982, but removing specifically bound sites, once attached, was more difficult. Given that approximately 10 μmol of ASCH was coupled to 1 mL of amine–agarose resin, the concentration of ASCH on the column was approximately 10 mM. Incubation with SCH-23390 (10 mM, a nearly saturated solution) removed only 50% of the total binding sites from the ASCH–agarose resin. This may have been due to the high concentration of ASCH on the resin. Altering the duration and number of incubation times with NaCl and SCH-23390 might change the amount of protein or binding sites eluted from the column.

The use of ASCH may also provide information with regard to the location and structure of D1 receptors. The coupling of ASCH to biotin hydrazide resulted in a molecule able to simultaneously bind D1 receptors through the ASCH portion of the molecule and avidin through the biotin moiety. D1 receptors on rabbit striatal membranes prebound with biotin–ASCH were detected by HRP-linked avidin along with a soluble HRP substrate. A fluorescent avidin compound could be used in the same manner. Fluorescein–SCH-23390 has been synthesized and used to detect D1 receptors in whole brain tissue sections (Ariano et al., 1989). A biotin-coupled derivative of SKF-83566-NH_2 has been synthesized but has not been shown to simultaneously couple to avidin and D1 receptors (Madras et al., 1990). Visualizing D1 receptors through biotin–ASCH may have an advantage over the use of fluorescein–SCH-23390. Signal amplification can be obtained by having several fluorescent molecules coupled to one molecule of avidin bound through biotin to a D1 receptor. Such amplification is not possible with a ligand that is covalently coupled to fluorescein. Biotin–ASCH should make it possible to analyze the cellular distribution of D1 receptors in the striatum and other brain regions and possibly the subcellular localization of the receptors. For example, biotin-linked ω-conotoxin has been used with colloidal gold to localize voltage-dependent calcium channels to dendrites of hippocampal CA1 neurons by electron microscopy (Jones et al., 1989).

The coupling of ASCH through its aldehyde moiety to large antigenic proteins, such as keyhole limpet hemocyanin, may permit the production of anti-idiotypic anti-receptor antibodies to aid in structural analysis of the binding site and the purification of D1 receptors. The β-adrenergic receptor was studied in this manner with antibodies to alprenolol used to make anti-idiotypic antibodies cross-reacting with the β-receptor (Sawutz et al., 1987). With an aldehyde moiety capable of being easily coupled to a variety of molecules and a selectivity for D1 receptors, ASCH should be a valuable tool in further studies.

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