benzimidoyl chloride, 4513-27-3; 2-(2-oxopyrrolidinyl)ethanol, 3445-11-2; 2-piperidinylethanol, 3040-44-6; 1-methyl-4-piperidinol, 106-52-5.

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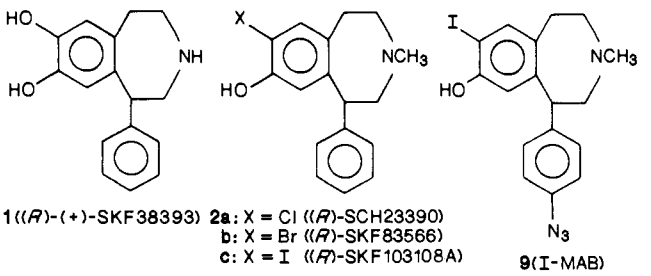
A Photoaffinity Label for the D-1 Dopamine Receptor, (*RS*)-7-[^{125}I]Iodo-8-hydroxy-3-methyl-1-(4′-azidophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine, Selectively Identifies the Ligand Binding Subunits of the Receptor

Sir:

Dopamine is a well-recognized neurotransmitter in the CNS as well as at some peripheral sites. Kebabian and Calne[1] proposed the classification of dopamine receptors into two categories: the D-1 and D-2 receptors. While the D-2 receptor has been studied extensively at both the pharmacological and molecular level,[2-5] the same is not true for the D-1 receptor owing, in large part, to the limited availability of potent, biospecific, and selective compounds. With the development of azidoclebopride[6] and azido-NAPS[7] as photoaffinity labels for the D-2 receptor, the need arose for similar photoaffinity labels to identify the ligand binding subunits of the D-1 receptor.

The benzazepines provide the most useful selective pharmacological probes of the D-1 receptor.[8] SKF 38393 (**1**) is the most widely used selective D-1 receptor agonist.[9] The benzazepines also provide the only examples of currently available selective antagonists of the D-1 receptor.[10] Several 7-halogenated N-methylated analogues of SKF 38393 have been shown to be potent, selective, stereospecific antagonists of the D-1 DA receptor. The chloro (**2a**, SCH 23390), bromo (**2b**, SKF 83566), and iodo (**2c**, SKF 103108A) analogues are all highly potent and selective with the activity residing in the *R*-(+) enantiomer. (*R*)-(+)-SKF 103108A ((*R*)-(+)SCH 23982) has been radiolabeled with high specific activity and is now the radioligand of choice for identifying the D-1 receptor.[11]

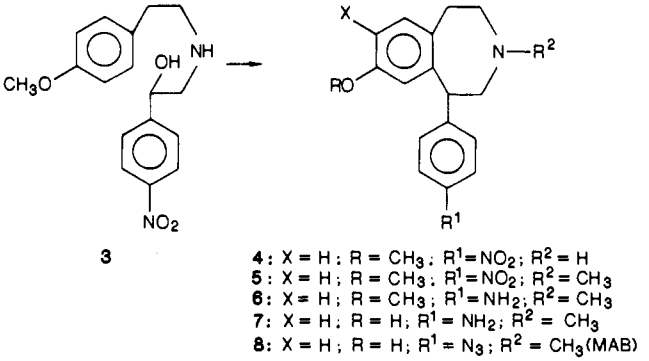
A retrospective structure-activity relationship (SAR) study of benzazepine agonists and antagonists indicated that the 1-phenyl ring could serve as the target for introduction of the amino and subsequently the azido functions for the development of a photoaffinity label. While this



1((*R*)-(+)-SKF38393) **2a**: X = Cl ((*R*)-SCH23390)
b: X = Br ((*R*)-SKF83566)
c: X = I ((*R*)-SKF103108A)

Figure 1.

Scheme I



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4: X = H; R = CH_3; R^1 = NO_2; R^2 = H
5: X = H; R = CH_3; R^1 = NO_2; R^2 = CH_3
6: X = H; R = CH_3; R^1 = NH_2; R^2 = CH_3
7: X = H; R = H; R^1 = NH_2; R^2 = CH_3
8: X = H; R = H; R^1 = N_3; R^2 = CH_3(MAB)

study was in progress, Caron et al.[12] reported an iodinated 3-aminophenyl derivative of SCH 23390 for use in the identification of the ligand binding subunit of rat striatal D-1 receptors (M_r = 72000) via photoaffinity cross-linking with *N*-succinimidyl 6-[(4-azido-2-nitrophenyl)amino]-hexanoate (SANPAH). Since it is advantageous to have a radioactive photoaffinity label, racemic SKF 103108A was chosen for the introduction of the amino and subsequently the azido functions. A synthetic route was devised that could provide a photoaffinity label of high specific activity by permitting iodination and hence radioiodination at the terminal step.

The general synthetic route established by Walter and Chang[13] for the construction of the 1-aryl-2,3,4,5-tetrahydro-1*H*-3-benzazepine skeleton was employed for the synthesis of the target compound. *p*-Nitrostyrene oxide was made by the method of Rafizadeh and Yates[14] in a one-pot synthesis starting from *p*-nitrobenzaldehyde. Condensation with 4-methoxyphenethylamine gave α-[[*N*-(4-methoxyphenyl)ethylamino]methyl]-4-nitrobenzyl alcohol (**3**) (mp 120-122 °C; 42% yield; $C_{17}H_{20}N_2O_4$: C, H, N). Attempted cyclization of **3** employing the standard sulfuric acid-TFA system failed in this case owing persumably to the destabilization of the incipient carbonium ion intermediate by the 4-nitro group. Cyclization with PPA proved successful and the required 8-methoxy-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (**4**) (mp 132-134 °C; 78% yield; $C_{17}H_{18}N_2O_3$: C, H, N) was obtained. N-Methylation of **4** was carried out by the standard Eschweiler-Clarke procedure[15] to obtain **5** (mp 116-118 °C; 84% yield; $C_{18}H_{20}N_2O_3$: C, H, N). This was reduced with Raney Ni and hydrazine hydrate in ethanol to yield **6** (mp 190-192 °C; 92% yield), which was subjected to O-demethylation with boron tribromide to **7** (mp 230-232 °C; 55% yield; $C_{17}H_{20}N_2O \cdot 2CH_3SO_3H \cdot 2H_2O$: C, H, N; *m/z* 268 ($M^+$)). Diazotization of **7** with sodium nitrite in 6 N sulfuric acid at 0 °C yielded the diazonium

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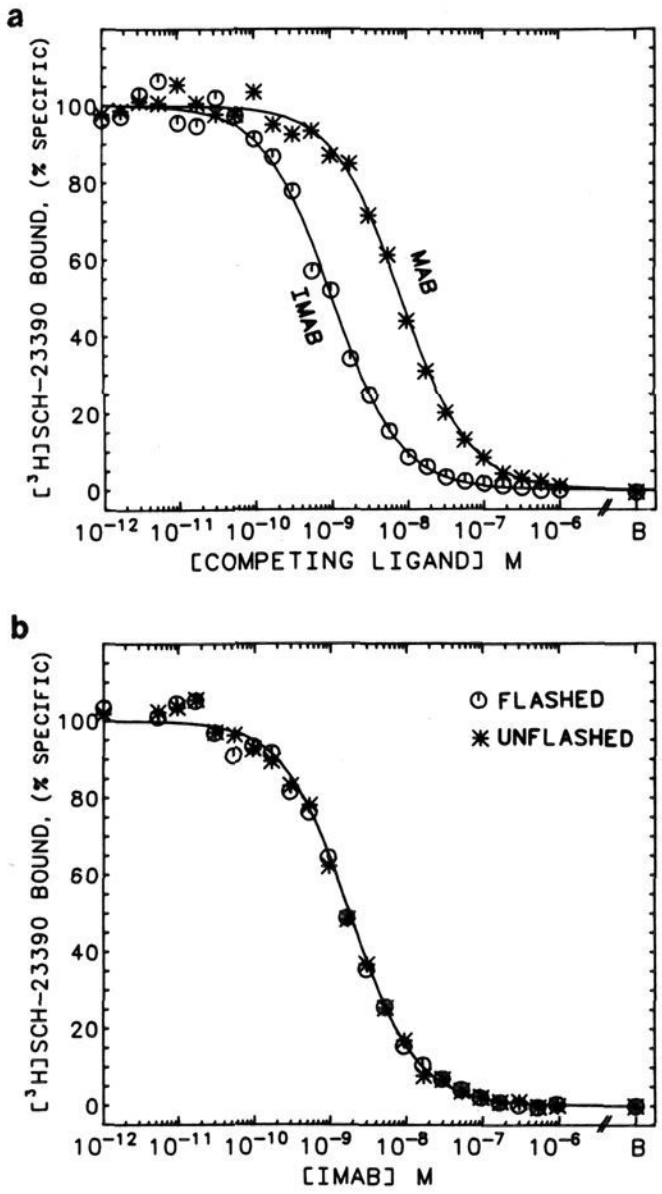

Figure 2. (a) Competition of [^{3}H]SCH 23390 binding to canine striatal D-1 receptors by I-MAB and MAB. Membranes were incubated with 200 pM [^{3}H]SCH 23390 and the indicated concentrations of competing ligands, in the dark, for 120 min at 22 °C and assayed for D-1 receptor activity. Data were analyzed by the nonlinear least-square curve-fitting program LIGAND with estimated K_D values listed in the text. Results are representative of three independent experiments. (b) Competition of [^{3}H]SCH 23390 binding to D-1 receptors by flashed and unflashed I-MAB. Stock solutions (3×10^{-4} M) of I-MAB were either exposed for 35 s to UV light (flashed) or kept in the dark (not flashed) and incubated with striatal membranes and 200 pM [^{3}H]SCH 23390 for 120 min, in the dark, and assayed for D-1 receptor activity. Data were analyzed by LIGAND. The experiment was repeated once with virtually identical results.

salt intermediate, which was immediately treated with sodium azide at the same temperature to yield **8** (mp 95–100 °C dec; 64% yield; $C_{17}H_{18}N_4O \cdot {}^1/_2H_2O$: C, H, N; m/z 294 (M^+), 266 ($M - N_2^+$)). Finally **8** was iodinated with ICl in acetic acid to obtain the iodo azido analogue I-MAB (**9**) (mp 175–180 °C; $C_{17}H_{17}IN_4O \cdot HCl \cdot {}^3/_2H_2O$: C, H, N; m/z 420 (M^+), 392 ($M - N_2^+$)). Radioiodination of **8** was carried out by using a modified Na^{125}I-chloramine T procedure and the product purified by reverse-phase HPLC to obtain the ^{125}I analogue of **9** in 50% radiochemical yield and with a specific activity of 2200 Ci/mmol. The full pharmacological characterization of [^{125}I]I-MAB as a selective photoaffinity probe for the D-1 receptor is described in a forthcoming publication.[16]

The ability of I-MAB (**9**) to interact with D-1 dopamine receptors was assessed in competition binding experiments with [^{3}H]SCH 23390. As depicted in Figure 2a, I-MAB inhibited the binding of the parent probe [^{3}H]SCH 23390

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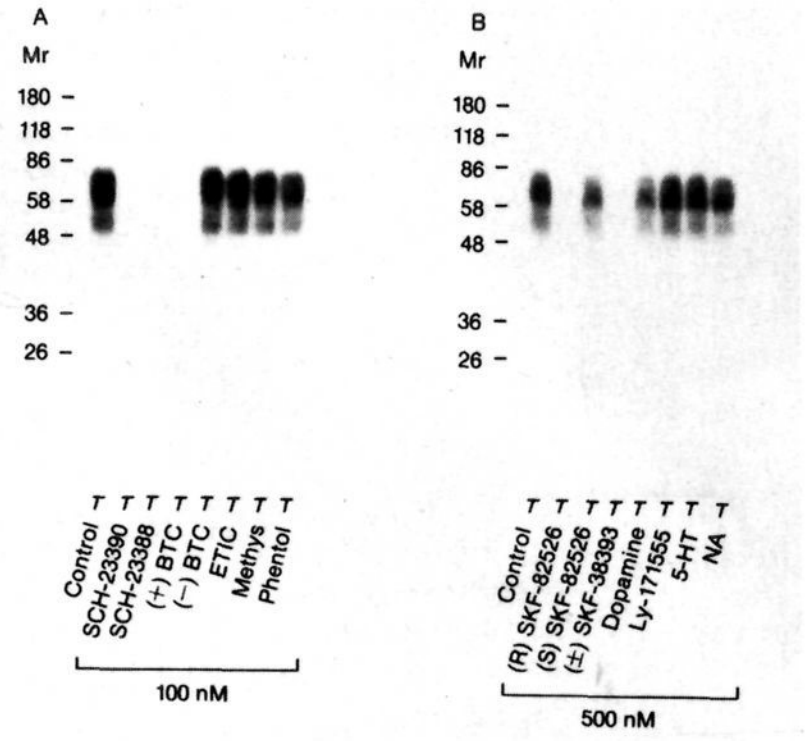

Figure 3. (A and B) Photoaffinity labeling and pharmacological specificity of [^{125}I]I-MAB photoincorporation into canine striatal membranes. Membranes were prepared and photoaffinity labeled with [^{125}I]I-MAB (200 pM) alone (control) or in the presence of the indicated concentrations of competing antagonists (A) or agonists (B). Samples were subjected to SDS-PAGE using 12% acrylamide gel and autoradiography. The M_r of known protein standards are shown ×1000. The results shown are representative of at least two similar experiments. Abbreviations used are (+)BTC, butaclamol; ETIC, eticlopride; METHYS, methysergide; PHENTOL, phentolamine; 5-HT, serotonin; NA, noradrenaline; SKF 82526, fenoldopam; LY 171555, quinpirole.

to canine striatal membranes in a concentration-dependent manner with a K_D of 380 pM and a Hill slope close to unity. The introduction of both an iodo and azido moiety only reduced the affinity of the ligand by 2-fold compared to that of SCH 23390 (K_D = 150 pM). Given the racemic nature of I-MAB (**9**), the K_D of this compound is probably closer to that of (*R*)-SCH 23390 than described here. Moreover, the ability of the precursor MAB (**8**) to inhibit [^{3}H]SCH 23390 binding is also shown (Figure 2a) with an estimated K_D value of 3.4 nM. It appears, therefore, that the introduction of the iodo and azido substituents does not directly interfere with binding to D-1 receptors.[16] As expected for a photoaffinity probe, preexposure of I-MAB to UV light does not interefere with the compound's ability to bind to D-1 receptors. As depicted in Figure 2b, prephotolyzed I-MAB inhibits [^{3}H]SCH 23390 binding to canine striatal membranes in a concentration-dependent manner with an observed K_D of 400 pM similar to that of I-MAB (K_D = 380 pM) which was not exposed to UV light. Moreover preliminary experiments have shown that I-MAB upon photolysis can inactivate D-1 receptors (as is indexed by subsequent [^{3}H]SCH 23390 binding) in a concentration-dependent manner with a pseudo IC_{50} of 10 nM (data not shown).

Photoaffinity Labeling of the Neuronal D-1 Dopamine Receptor. The major aim of this work was to develop an aryl azide derivative of SCH 23390 that can, upon photolysis, covalently incorporate into and identify the ligand binding subunit of D-1 receptors. Figure 3 depicts the results obtained when canine striatal membranes were incubated with [^{125}I]I-MAB and photolyzed and samples subjected to SDS-PAGE and autoradiography. A broad band was labeled at an apparent M_r = 75 000. The specificity of labeling is shown by virtue of the fact that covalent photoincorporation of [^{125}I]I-MAB into the M_r = 75 000 subunit was blocked by 100 nM SCH

23390. In addition, two distinct specifically labeled bands at apparent M_r = 62 000 and 51 000 were seen within the diffuse pattern of labeling.

The pharmacological specificity of [^{125}I]I-MAB photoincorporation was assessed by examining the ability of various dopaminergic agents to block covalent labeling of these subunits of the D-1 receptor in canine striatal membranes. As seen in Figure 3, the photolysis-dependent covalent labeling of the subunits at M_r = 75 000, 62 000, and 51 000 was stereoselectively blocked by the active isomers of SCH 23390 and butaclamol (Figure 3A). In contrast, incubation of [^{125}I]I-MAB with a selective D-2 receptor antagonist (eticlopride), serotonin S_2 receptor antagonist (methysergide), or adrenergic receptor antagonist (phentolamine) did not prevent the covalent incorporation of [^{125}I]I-MAB into these subunits. The ability of SCH 23388 (the *S*-(-) enantiomer of SCH 23390) to partially antagonize the covalent labeling of these three ligand binding subunits is a reflection of its high affinity for the receptor (K_i = 20 nM).[17] Virtually identical results were obtained when canine striatal membranes were incubated with [^{125}I]I-MAB, in the presence of dopaminergic agonists, photolyzed, and subjected to SDS-PAGE (Figure 3B). Covalent incorporation of [^{125}I]I-MAB was stereoselectively blocked by (*R*)-SKF 82526 (fenoldopam) but not by (*S*)-SKF 82526 at a concentration of 500 nM. Similarly, the selective D-1 agonist (*RS*)-SKF 38393 prevented the incorporation of [^{125}I]I-MAB into the polypeptides of apparent M_r = 75 000, 62 000, and 51 000, while the selective D-2 receptor agonist LY 171555 (quinpirole), the adreneric agent noradrenaline, or the serotonergic agent serotonin did not.

Taken together these data strongly suggest that the peptides at apparent M_r = 75 000, 62 000, and 51 000 are the ligand binding subunits of the neuronal D-1 receptor. The exact molecular relationship among these subunits is unknown at present and could represent proteolytic degradation products of the M_r = 75 000 protein, but the inclusion of mutliple class specific protease inhibitors previously shown to be useful in preventing receptor degradation in other systems did not prevent the specific labeling of these lower molecular weight species.[16] Moreover, the mechanism regulating the inefficiency of dopamine (500 nM) at inhibiting [^{125}I]I-MAB incorporation into these peptides is unknown, but previous work has shown that [^{125}I]I-MAB labeling is blocked at higher concentrations of dopamine (5 μM) and still displays an appropriate rank order of potency for dopamine receptors.[16] In any event, the development of a high-affinity, high specific activity photoaffinity label for the D-1 receptor should aid in the subsequent molecular characterization of this protein in numerous tissues and under various experimental conditions.

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Registry No. **3**, 116351-49-6; **4**, 116234-46-9; **5**, 116234-47-0; **6**, 116263-67-3; **7**, 116234-48-1; **7**·2CH_3SO_3H, 116351-50-9; **8**, 116234-45-8; **9**, 116234-50-5; **9**·HCl, 116351-51-0; **9** (^{125}I), 116234-44-7; *p*-nitrostyrene oxide, 6388-74-5; 4-methoxyphenethylamine, 55-81-2.

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