treatment. The receptor-dependent clearance of plasma LDL is also significantly increased in FH heterozygotes by mevinolin treatment.[46] Thus, LDL lowering in these patients can be explained by the increased LDL receptor activity.

Conclusion. In the Japanese population, as well as the European and American populations, FH occurs at a frequency of 1 in 500. It is closely associated with premature atherosclerotic heart disease. The increased levels of LDL in FH is believed to accelerate atherosclerosis.

FH is a disorder characterized by the defect in LDL receptor, which causes a decrease in LDL uptake by the cell. The decreased LDL uptake, in turn, results in higher levels of both plasma LDL and cellular HMG-CoA reductase activity. Thus, treatment should increase LDL catabolism by stimulating the LDL pathway. As mentioned, treatment with compactin (or mevinolin) is ideal in FH in that it reduces plasma LDL levels by stimulating the LDL receptor-mediated catabolism.

Of the analogues related to compactin, the acid form of 3β-hydroxycompactin (CS-514) (Figure 2), which is comparable to compactin in both in vitro and in vivo activities, has recently been reported to be superior to the parent compound in safety.[47] Compactin-related compounds have not been widely used in the treatment of hypercholesterolemia. Yet the studies with these agents have established a general principle: a competitive inhibitor of HMG-CoA reductase can reduce LDL levels in plasma by increasing LDL receptor without depleting vital body stores of cholesterol.

Registry No. HMG-CoA reductase, 9028-35-7; compactin, 73573-88-3; cholesterol, 57-88-5.

(47) S. Fujii, M. Arai, Y. Tsujita, and M. Tanaka, presented at the 16th General Meeting of the Japanese Society of Atherosclerosis (Tokyo), July 22–23, 1984.

Communications to the Editor

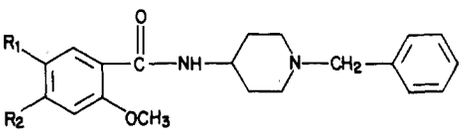
Novel Photoaffinity Label for the Dopamine D_2 Receptor: Synthesis of 4-Azido-5-iodo-2-methoxy-*N*-[1-(phenylmethyl)-4-piperidinyl]benzamide (Iodoazidoclebopride, IAC) and the Corresponding ^{125}I-Labeled Analogue (^{125}IAC)

Sir:

Dopamine agonist elecited behaviors (rotation, psychotomimetic actions, antiparkinsonian action, stereotypy, and locomotion) appear to be mediated by dopamine D_2 receptors in the brain.[1]

Although the dopamine D_2 receptor has been solubilized by several laboratories,[2] attempts to isolate and purify the protein by affinity chromatography[3] or photoaffinity labeling[4,5] have been relatively unsuccessful.[6,7] Alkylating type irreversible ligands have been developed (i.e., [^{3}H]-*N*-(chloroethyl)norapomorphine, phenoxybenzamine, *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline, flupenthixyl chloride), but have been too low in receptor affinity and/or selectivity to be of value in dopamine D_2 receptor isolation.[3,8-10] The molecular characterization of dopamine D_2 receptors has been hampered by the lack of specific photoaffinity probes which can be used to covalently label these sites.

We report here the synthesis of a series of substituted benzamides (Ib–Ig) and in particular Ig, an agent, useful as a photoaffinity label, which selectively and irreversibly labels dopamine D_2 receptors upon light irradiation.



Ia, R_1 = Cl; R_2 = NH_2; clebopride
Ib, R_1 = Cl; R_2 = N_3; azidoclebopride (AC)
Ic, R_1 = H; R_2 = NH_2;
Id, R_1 = I; R_2 = NH_2; iodoclebopride
Ie, R_1 = I; R_2 = N_3; iodoazidoclebopride (IAC)
If, R_1 = ^{125}I; R_2 = NH_2; [^{125}I]iodoclebopride
Ig, R_1 = ^{125}I; R_2 = N_3; [^{125}I]iodoazidoclebopride (^{125}IAC)

Since clebopride (Ia) was reported as a selective D_2 receptor antagonist,[11-13] we selected the substituted benzamide as a ligand that has inherent affinity for the binding site of D_2 receptors and incorporated an azido group as a photosensitive functional group, replacing the amino group in Ia. When Ib was photoactivated with light, it was capable of forming a covalent bond at or near the binding site.[14,15] The association with the recognition site will ordinarily be reversible until photolysis is initiated; the covalent linkage thus formed between the photoprobe and the binding site will thus facilitate the characterization and isolation of the dopamine D_2 receptor.

Clebopride (Ia) when reacted with sodium nitrite and concentrated hydrochloric acid formed the intermediate diazonium salt which was treated with an aqueous solution

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Table I. Protection of [^{3}H]Spiperone Binding Activity against Photoinactivation with Iodoazidoclebopride[a]

agonists	IC_{50},[a] nM
(−)-*N*-*n*-propylnorapomorphine	1×10^{-9}
(+)-6,7-dihydroxy-2-aminotetralin	4×10^{-8}
(+)-*N*-*n*-propylnorapomorphine	1×10^{-6}
dopamine	8×10^{-6}
norepinephrine	8×10^{-5}
serotonin	3×10^{-4}
antagonists	**IC_{50}, nM**
spiperone	3×10^{-10}
(+)-butaclamol	5×10^{-9}
(*S*)-(−)-sulpiride	2×10^{-8}
(−)-butclamol	$>10^{-5}$

[a] Canine striatal membranes were incubated with IAC (Ie) (30 nM) and varying concentrations of protecting drug for 90 min under reduced light, irradiated for 35 s (85 W, Hg lamp), and extensively washed as described in ref 18. Dopamine D_2 receptor activity was assayed as described. IC_{50} is the concentration of drug required to inhibit dopamine D_2 receptor photoinactivation by 50%. [b] Data derived from ref 18 and represent the means obtained from one to three independent experiments with a SEM <8%.

of sodium azide, to yield azidoclebopride (Ib), mp 200–201 °C dec (from absolute EtOH), 42% yield; MS, *m/z* 399. Irradiation of canine striatal membranes in the presence of azidoclebopride (see ref 15 for experimental details) reduced the specific binding of [^{3}H]spiperone[16] in a concentration-dependent manner with a pseudo IC_{50} of 80 nM. The ability of azidoclebopride to photoinactivate D_2 receptors was markedly reduced in the absence of sodium chloride (pseudo IC_{50} = 4000 nM). Maximal photoinactivation was attained with 1 μM azidoclebopride (Ib) and typically represented a loss of 60% of the total number of binding sites for [^{3}H]spiperone. The data demonstrated that dopamine D_2 receptors can be photoinactivated by azidoclebopride and protection against photolysis is provided by dopaminergic agonists with an appropriate pharmacological profile: (−)-*N*-*n*-propylnorapomorphine > apomorphine > (±)-6,7-dihydroxy-2-aminotetralin > (+)-*N*-*n*-propylnorapomorphine > dopamine > norepinephrine > serotonin.[14,15]

Thus it was speculated that the incorporation of a radioactive moiety such as ^{125}I into azidoclebopride may yield a photoactive ligand of sufficiently high specific activity and affinity to be of use in the isolation of the D_2 dopamine receptor. The direct incorporation of iodine onto Ia was unsuccessful. Thus we substituted the chlorine atom in Ia with iodine, although the chlorine atom was reported as the optimal substituent among the piperidylbenzamides evaluated.[17]

The synthesis of iodoclebopride (4-amino-5-iodo-2-methoxy-*N*-[1-(phenylmethyl)-4-piperidinyl]benzamide (Id) was achieved by the condensation of methyl 4-(acetylamino)-2-methoxybenzoate and 4-amino-1-benzylpiperidine in the presence of aluminum isopropylate in xylene at 80 °C, yielding 4-(acetylamino)-2-methoxy-*N*-[1-(phenylmethyl)-4-piperidinyl]benzamide in 57% yield, mp 171–174 °C; MS, *m/z* 381. Subsequent hydrolysis with 12% HCl gave Ic (51%; mp 189 °C dec; MS, *m/z* 339). Iodination of Ic was accomplished with iodine monochloride in glacial acetic acid to yield (Id) (iodoclebopride) in quantitative yield (mp 146–147 °C; MS, *m/z* 465). Further treatment of Id with sodium nitrite and sodium azide yielded, after recrystallization from ethanol, Ie (iodoazidoclebopride) in 85% yield (mp 195–197 °C dec; MS, *m/z* 491). Iodoazidoclebopride retained high affinity for canine striatal dopamine D_2 receptors with a dissociation constant (K_D) of 14 nM. Irradiation of a striatal homogenate with Ie irreversibly inactivated 50% of dopamine D_2 receptors[18] at 20 nM (as indicated by subsequent [^{3}H]spiperone binding). Dopamine agonists and antagonists prevented this photoinactivation with the appropriate rank order of potency (Table I). Inactivation of D_2 receptors with iodoazidoclebopride did not alter the ability of guanyl nucleotides and sodium ions to lower the affinity of the D_2 receptor for dopamine agonists (Table II). Thus, iodoazidoclebopride (Ie) was demonstrated to be a selective photoaffinity probe for dopamine D_2 receptors.

The conversion of cold Id to [^{125}I]iodoclebopride (If) was carried out by iodine exchange with use of sodium [^{125}I]-iodide with carrier (14 Ci/mmol). Extraction from ether, evaporation to dryness, and further treatment with sodium nitrite and hydrochloric acid, followed by direct addition of sodium azide to the reaction mixture, gave Ig, which was

Table II. Guanyl Nucleotide and Sodium Regulation of D_2 Dopamine Receptors[a]

treatment		K_D for ADTN, nM	
		control	Gpp(NH)p/NaCl
UV alone	D_2 high	12 ± 0.1	281 ± 36
	D_2 low	7000 ± 830	10981 ± 1200
UV + IAC	D_2 high	14 ± 0.4	267 ± 43
	D_2 low	11000 ± 1500	15650 ± 1900

[a] Canine striatal membranes were incubated in the presence or absence of IAC (30 nM, final concentration) for 90 min (22 °C) in Tris-HCl buffer (containing 120 mM NaCl) under reduced light, irradiated for 35 s, and extensively washed in Tris-HCl–0.5% bovine serum albumin buffer (sodium-free) as described.[17] Aliquots of washed brain membranes were incubated with varying concentrations of (±)-6,7-dihydroxy-2-aminotetralin (ADTN) and 80 pM [^{3}H]spiperone in the absence (control) or presence of 100 μM 5-guanylylimidodiphosphate (Gpp(NH)p) and 120 mM NaCl for 45 min at 37 °C. Incubations were terminated by rapid filtration as previously described.[15,18] Data were analyzed by the nonlinear curve fitting computer program LIGAND. The experiment was repeated with similar results.

Table III. Tissue Distribution of (Percent Dose/Gram ± SD) in Rats after Intravenous Administration of ^{125}IAC and [^{125}I]Iodoclebopride (If)

	^{125}IAC		[^{125}I]iodoclebopride	
	1 h	2 h	1 h	2 h
blood	0.32 ± 0.03	0.34 ± 0.05	0.33 ± 0.06	0.29 ± 0.05
heart	1.61 ± 0.03	1.34 ± 0.1	0.35 ± 0.03	0.30 ± 0.04
cerebellum	1.13 ± 0.18	1.06 ± 0.07	0.26 ± 0.03	0.23 ± 0.01
thalamus	1.25 ± 0.32	1.12 ± 0.15	0.27 ± 0.05	0.22 ± 0.03
striatum	2.52 ± 1.51	2.52 ± 0.64	0.5 ± 0.11	0.38 ± 0.18
rest of brain	0.78 ± 0.10	0.73 ± 0.06	0.18 ± 0.01	0.16 ± 0.02
striatum/cerebellum	2.23	2.37	1.92 ±	1.65

(16) [^{3}H]Spiperone binding to D_2 dopamine receptors was measured as previously described.[15] Briefly, aliquots (0.5 mL) of washed striatal membranes were incubated with 0.75 mL of Tris-HCl buffer (containing: 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, and 120 mM NaCl, pH 7.4 at 37 °C) and 1 nM [^{3}H]spiperone (final concentration) in a final volume of 1.5 mL, for 45 min at 37 °C. Incubations were rapidly terminated by filtration (Titertek Cell Harvester) through glass-fiber filter mats. Filters were rinsed for 15 s (10 mL) with 50 mM Tris-HCl buffer, pH 7.4, at 22 °C, placed in scintillation vials containing Scint-A scintillation fluid, and monitored for tritium in a Packard 460 C scintillation spectrometer at 43% efficiency. The binding in the presence of 1 μM (+)-butaclamol was defined as nonspecific.

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isolated by extracting with ether and washing several times with distilled water. The product radioactivity of Ig was homogeneous on TLC (14 Ci/mmol) and coeluted with Ie. Elemental analysis for all new compounds were within 0.4% of theoretical values. ^{1}H NMR, IR, and UV spectra were consistent with the assigned structures.

The biodistribution of [^{125}I]iodoclebopride (If) and [^{125}I]iodoazidoclebopride (Ig) was evaluated in rats. CD Fisher rats (150–200 g) were injected via the tail vein with a slightly acidic saline solution of ^{125}IAC (Ig) and [^{125}I]-iodoclebopride (If). The animals were sacrificed (six for each time point) with ether and the biodistribution was carried out by sampling the organ and brain sections and counting. The uptake was calculated as a percent dose injected per gram (Table III). In the case of [^{125}I]iodo-clebopride (If), the striatal uptake expressed in percent injected dose per gram was 0.5 at 1 h and decreased to 0.38 at 2 h. The striatum to cerebellum ratio decreased from 1.92 at 1 h to 1.65 at 2 h. Whereas, in the case of ^{125}IAC (Ig), the striatal uptake expressed in percent injected dose per gram was 2.52 and remained the same after 2 h. The striatum to cerebellum ratio increased slightly from 2.23 at 1 h to 2.37 at 2 h.

The binding behavior of [^{125}I]iodoclebopride was found to be comparable to haloperidol.[19] However, our results indicate high affinity for ^{125}IAC (Ig) to striatal dopamine receptors which lasts for at least 2 h. The mechanism for this sustained in vivo binding suggesting irreversible covalent binding to the receptor without UV activation remains to be clarified. These observations and our present results point to the potential use of ^{125}IAC as an agent for the in vitro and in vivo characterization of D_2 receptors.

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