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Haloperidol-succinylglycyl[^{125}I]iodotyrosine, a novel iodinated ligand for dopamine D_2 receptors

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A novel radioiodinated ligand of the butyrophenone type has been synthesized for the quantification and characterization of dopamine D_2 receptors. This haloperidol-derived ligand, haloperidol-succinylglycyl-[^{125}I]iodotyrosine ([^{125}I]HSGTI), binds rapidly (equilibrium is reached within 30 min, at 10 pM and 37 °C) and with high affinity ($K_d = 0.3$ nM) to bovine striatal membranes. Its pharmacology, determined by competitive displacement with dopaminergic and non-dopaminergic drugs, is characteristic of binding to dopamine D_2 receptors.

Dopamine D_2 receptor; Radioiodinated ligand; (Striatum)

1. INTRODUCTION

Until recently, no iodinated compound had been proposed for the investigation of dopaminergic receptors. Several tritiated ligands have been used to label them, allowing knowledge to be gained concerning their pharmacology. The results obtained from binding experiments using a great variety of antagonist and agonist molecules, correlated with functional data (adenylate cyclase activity), have led to the definition of two classes of dopaminergic receptors, termed D_1 and D_2 [1,2]. Both classes display two states of agonist affinity [3–5]. However, during the last 3 years, three iodinated drugs have been developed: ^{125}I-sulpride [6], a benzamide derivative, and two butyrophenone derivatives, ^{125}I-spiperone [7] and ^{125}I-NAPS [8]. ^{125}I-NAPS is a convenient covalent photoaffinity probe. Here, we present the development of another iodinated compound of the butyrophenone class: haloperidol-succinylglycyl-[^{125}I]iodotyrosine ([^{125}I]HSGTI), and its characterization using bovine striata. This iodinated derivative was devised not only as a ligand of dopaminergic receptors, but also as a radioactive tracer for the radioimmunoassay (RIA) of small amounts of haloperidol in small brain tissue samples and in serum samples (unpublished).

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2. MATERIALS AND METHODS

The radioactive materials used in these studies were from commercial sources: Na^{125}I (carrier-free) and [^{3}H]spiperone (1 TBq/mmol) were obtained from New England Nuclear (Dreieich, FRG). Most of the drugs were generous gifts from laboratories: Janssen Pharmaceutica (Beerse, Belgium), Lundbeck (Copenhagen, Danmark), Organon (Oss, The Netherlands) and SKF (Welwyn, England). Dopamine was from Sigma (St. Louis, USA), and butaclamol from Ayerst Laboratories (Montreal, Canada). All given pH values were determined at 25°C.

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2.1. *[^{125}I]HSGTI synthesis*

The formation of haloperidol-hemisuccinate has been described elsewhere [9]. The coupling to glycyltyrosine was achieved as follows: haloperidol-hemisuccinate (10 μmol) in 1 ml acetonitrile containing triethylamine (20 μmol) was activated with ethylchloroformate (20 μmol) for 5 min at 4°C. 1 ml of a solution of glycyl-L-tyrosine (50 μmol) in acetonitrile/0.2 M triethylamine (50:50) was added to the activated solution with stirring. After 15 min, the mixture was purified by HPLC on a C_{18} column, using acetonitrile/10 mM ammonium acetate, pH 6.2 (50:50), as the mobile phase. The relevant haloperidol-succinylglycyltyrosine (HSGT) fractions were pooled, the acetonitrile evaporated and the resulting solution freeze-dried. HSGTI was obtained by iodination of HSGT by a standard chloramine T procedure and purified by HPLC on a C18 column, using acetonitrile/10 mM potassium phosphate, pH 6.2 (40:60), as the solvent system. HSGT and HSGTI were identified by UV spectrophotometry by comparing their spectra with those of solutions containing a mixture of the same amounts of haloperidol and glycyltyrosine, or of haloperidol and glycyliodotyrosine. The radioactive compound was synthesized from HSGT by the same procedure. In this case, 10 μCi $Na^{125}I$ and 10 nmol chloramine T were added to 1 nmol HSGT in 50 mM potassium phosphate buffer, pH 7.2. The reaction was stopped after 1 min by adding 50 nmol metabisulfite. The monoiodinated compound [^{125}I]HSGTI was identified by HPLC by comparing its retention time with that of HSGTI. The purified fraction was diluted to 600000 cpm/ml in 50 mM Tris-HCl, pH 7.4, and stored at −20°C. Due to the complete separation of the iodinated derivative from $Na^{125}I$ and HSGT, the specific activity of [^{125}I]HSGTI was assessed as 2175 Ci/mmol.

2.2. *Bovine striatal membranes*

Fresh bovine brains were obtained from a local slaughterhouse. Striata were removed as rapidly as possible and homogenized in 10 vols (w/v) of ice-cold 10 mM Tris-HCl, pH 7.4, containing 320 mM sucrose and 10 mM $MgCl_2$. The homogenate was centrifuged at 1000 × g for 10 min and the resulting supernatant was spun at 27000 × g for 30 min. The resulting pellet was resuspended in ice-cold 50 mM Tris-HCl, pH 7.4, and stored at −20°C.

2.3. *Binding studies*

Incubations were carried out, in a total volume of 250 μl, in 50 mM Tris-HCl, pH 7.4, 30 mM $MgCl_2$ (unless stated otherwise). The incubation media contained [^{125}I]HSGTI at the appropriate concentration, the membrane preparation (approx. 25 μg protein) with or without 1 μM (+)-butaclamol (to define non-specific binding), or with various concentrations of different competitors. After incubation at 37°C for 40 min (or for the indicated times), the bound ligand was separated from the free ligand by centrifugation at 8700 × g for 1 min at 4°C. Aliquots of the supernatant were taken to estimate the free ligand. The pellet was superficially washed with 1 ml ice-cold buffer and recentrifuged. The bottom of the tubes containing the pellets were cut before counting.

No hydrolysis of [^{125}I]HSGTI was detected by HPLC during the incubation (30 min) with brain membranes. Experimental data were fitted using a Hewlett-Packard HP 9845 calculator and computing programs developed in the laboratory [10]. The curves were fitted using all individual data, except for the competition curves (12–16 concentrations of displacing drug) represented in fig.3, which were drawn using the mean of the triplicate values for each concentration.

3. RESULTS AND DISCUSSION

3.1. *Kinetics and equilibrium binding data*

The binding of [^{125}I]HSGTI was linear over a large range of protein concentrations: 20–500 μg/ml. [^{125}I]HSGTI bound rapidly to bovine striatal membranes, reaching equilibrium within 30 min at 10 pM and within 6 min at 4 nM (fig.1A). Binding was then stable for at least 60 min. Specific binding represented 60% of the total binding at 10 pM, and 40% at 1 nM. The association rate constant k_1 was determined to equal 0.12×10^9 $mol^{-1} \cdot min^{-1}$. The dissociation rate was also examined (fig.1B). The dissociation rate constant k_{-1} was determined to be equal to 0.06 min^{-1}, with a $t_{1/2}$ = 12 min. The equilibrium dissociation constant deduced from these kinetic data was $K_d = k_{-1}/k_1$ = 0.50 nM. This constant was also determined by equilibrium binding as seen

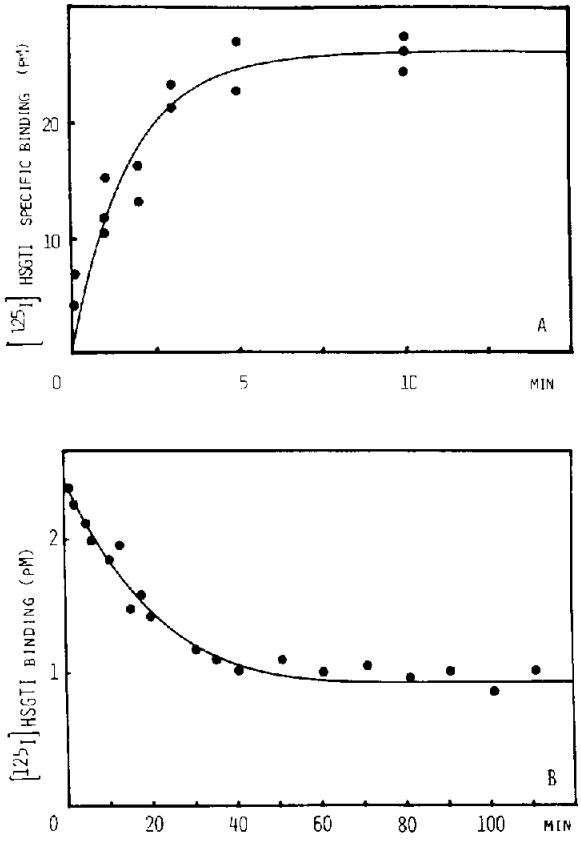

Fig.1. Kinetics of [^{125}I]HSGTI binding to bovine striatal membranes. Membranes were incubated as described in section 2, with [^{125}I]HSGTI at 4 nM, for the association kinetics (A), and 40 pM, for the dissociation kinetics (B). At the indicated times, samples were centrifuged for 1 min at 4°C to measure bound ligand. In B, the dissociation was initiated, after an equilibration of 30 min with [^{125}I]HSGTI, by the addition of 1 μM (+)-butaclamol to the incubation mixtures.

in fig.2. The equilibrium parameters derived from the experimental values were K_d = 0.30 ± 0.04 nM and B_{max} = 192 ± 15 fmol/mg protein. The dissociation constants obtained by both procedures were of the same order of magnitude.

The optimum specific binding was observed only in the presence of 20–30 mM divalent cation (Ca^{2+}, Mg^{2+}, or Mn^{2+}). The monovalent cations, K^+ and Li^+, were less effective in potentiating specific binding, while Na^+ even inhibited it, contrasting with its action on sulpiride binding (table 1) [12].

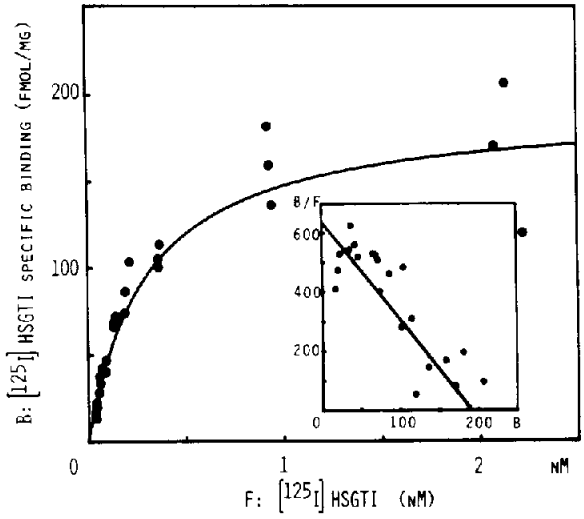

Fig.2. Saturation binding isotherm for [^{125}I]HSGTI. Bovine striatal membranes (25 μg) were incubated as described in section 2 with increasing concentrations of [^{125}I]HSGTI. Incubations were carried out at 37°C for 15 min. (Inset) Scatchard plot of the data.

Table 1

Dissociation constants of dopaminergic and non-dopaminergic drugs

Drugs	Inhibition constants K_i (nM)
Haloperidol	2 ± 0.5
Spiperone	0.6 ± 0.1
(+)-Butaclamol	0.9 ± 0.1
(−)-Butaclamol	>10000
Domperidone	2 ± 0.5
Sulpiride	1590 ± 318
Sulpiride + 100 mM NaCl	190 ± 75
Flupentixol	23 ± 8
SCH 23390	4250 ± 1700
SKF 38393	1074 ± 260
Dopamine	2760 ± 520
Mianserine	>40000
Alprenolol	>30000
Prazosine	1700 ± 600
Yohimbine	2500 ± 1000

[^{125}I]HSGTI (40 pM) was used to determine IC_{50} values. Because of the low concentration of the tracer, K_i values were considered to be close to the IC_{50} values

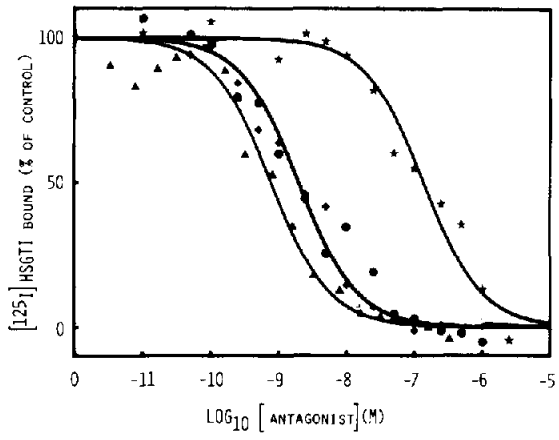

Fig.3. Competition curves of a series of dopaminergic antagonists for the binding of [^{125}I]HSGTI (40 pM) to bovine striatal membranes: (●) domperidone, (+) haloperidol, (▲) spiperone, (★) sulpiride. Binding is expressed as the percentage of the specific binding of controls which, depending on the experiment, corresponded to 3–6 pM bound [^{125}I]HSGTI.

3.2. *Specificity of [^{125}I]HSGTI binding*

Competition experiments with dopaminergic D_2 antagonists are illustrated in fig.3 and table 1. The rank order of potency observed for the displacement of [^{125}I]HSGTI by these compounds was similar to that described for [^{3}H]spiperone [2] and [^{3}H]haloperidol [13,14] displacements: spiperone > (+)-butaclamol > domperidone > haloperidol > sulpiride > (−)-butaclamol. The ligands specific for the D_1 receptors, such as SCH 23390 and SKF 38393 [15], had low affinities for [^{125}I]HSGTI-binding sites. Likewise, ligands specific for serotoninergic (mianserin), β-adrenergic (alprenolol), or α_2-adrenergic (yohimbine) receptors were inefficient in displacing [^{125}I]HSGTI from bovine striatal membranes. Haloperidol has been described as competing with high affinity for α_1-adrenergic sites [16]. However, its new iodinated derivative did not seem to bind to these sites (at least at the concentrations explored) since prazosin displaced [^{125}I]HSGTI binding only in the micromolar concentration range despite known binding to α_1-adrenergic sites in the nanomolar range [17].

This novel iodinated ligand therefore offers good D_2 dopaminergic specificity. The coupling of haloperidol to the dipeptide glycyltyrosine does not alter the affinity of the neuroleptic for dopaminergic sites. A similar observation was made by Jacobson et al. [18] for a series of derivative antagonists of adenosine receptors, obtained by covalent binding of amino acids to a xanthine backbone. The high specific activity of [^{125}I]HSGTI should allow the measurement of D_2-binding sites in small membrane samples prepared either from cultured cells, or from punches of brain tissue. Data obtained from measurements in samples taken from the central nervous system, combined with the RIA determination of haloperidol levels in equivalent material using the same ligand, should allow the direct comparison of receptor densities and haloperidol distributions in different brain regions following, for instance, an experimental treatment of rats with this neuroleptic.

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